Survival of C.elegans with three different media

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code for fitting Kaplan-Meier and log-rank test and for displaying survival curves for each type of media and mutant.

```
data <- read.table(here('data','Worm_mutant.csv'), header = TRUE, sep = ";", dec = ",")
head(data)
```

```
##   ï..Time Status Replicate Group Mutant
## 1       4      1        NA  FG13    PMK
## 2       6      1        NA  FG13    PMK
## 3       8      1        NA  FG13    PMK
## 4       8      1        NA  FG13    PMK
## 5      10      1        NA  FG13    PMK
## 6      10      1        NA  FG13    PMK
```

here() should show that your position is in the folder 7.semester else select the active project to be 7.semester in the upper right corner.

Or make a new .Rproj file in the folder 7.semester. There is a problem with the numbers of the worms so we change that with col.names()

```
colnames(data)<-c("Time","status",'Replicate', 'media',
                  'mutant')
head(data)
```

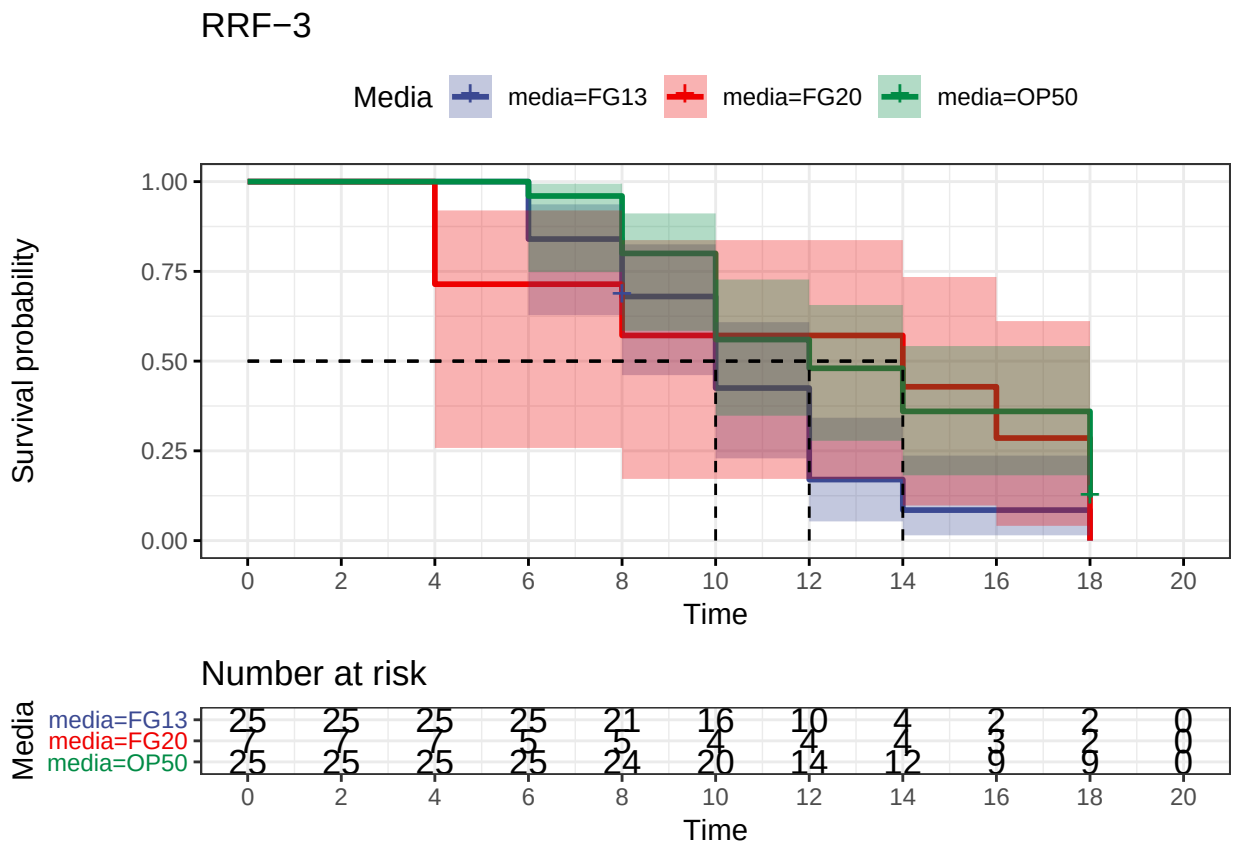
```
##   Time status Replicate media mutant
## 1    4      1        NA  FG13    PMK
## 2    6      1        NA  FG13    PMK
## 3    8      1        NA  FG13    PMK
## 4    8      1        NA  FG13    PMK
## 5   10      1        NA  FG13    PMK
## 6   10      1        NA  FG13    PMK
```

Then we create a survival object with the survival package and make a kaplan-meier curve

First we see for each mutant

```
d_RRF <- subset(data, mutant == 'RRF')
d_PMK <- subset(data,mutant == 'PMK')
d_DAF <- subset(data, mutant == 'DAF')
```

```
d_RRF$Survobj <- with(d_RRF,Surv(d_RRF$Time, event = d_RRF$status))
km_RRF <- survfit(Survobj ~ media, data = d_RRF,conf.type = "log-log", error = "greenwood")
s_km_RRF <- summary(km_RRF)
ggsurvplot(km_RRF,data = d_RRF, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'RRF-3')
```




```
df_fly_13_r <- data.frame(c(0,s_km_RRF$time[1:6]),c(1,s_km_RRF$surv[1:6]),                    c(0,s_
colnames(df_fly_13_r) <- c('Time','Surv','Std.error')
df_fly_20_r <- data.frame(c(0,s_km_RRF$time[7:11])
                          ,c(1,s_km_RRF$surv[7:11]),    c(0,s_km_RRF$std.err[7:11]))
colnames(df_fly_20_r) <- c('Time','Surv','Std.error')
df_OP50_r <- data.frame(c(0,s_km_RRF$time[12:17])
                        ,c(1,s_km_RRF$surv[12:17]),
                          c(0,s_km_RRF$std.err[12:17]))
colnames(df_OP50_r) <- c('Time','Surv','Std.error')

plot(df_fly_13_r$Time[2:7],df_fly_13_r$Surv[2:7], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13_r$Time,df_fly_13_r$Surv)
arrows(df_fly_13_r$Time, df_fly_13_r$Surv-df_fly_13_r$Std.error, df_fly_13_r$Time,
       df_fly_13_r$Surv+df_fly_13_r$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13_r$Time, df_fly_13_r$Surv -
## df_fly_13_r$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

```
points(df_OP50_r$Time[2:9],df_OP50_r$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50_r$Time,df_OP50_r$Surv, lty = 2)
arrows(df_OP50_r$Time, df_OP50_r$Surv-df_OP50_r$Std.error,
```

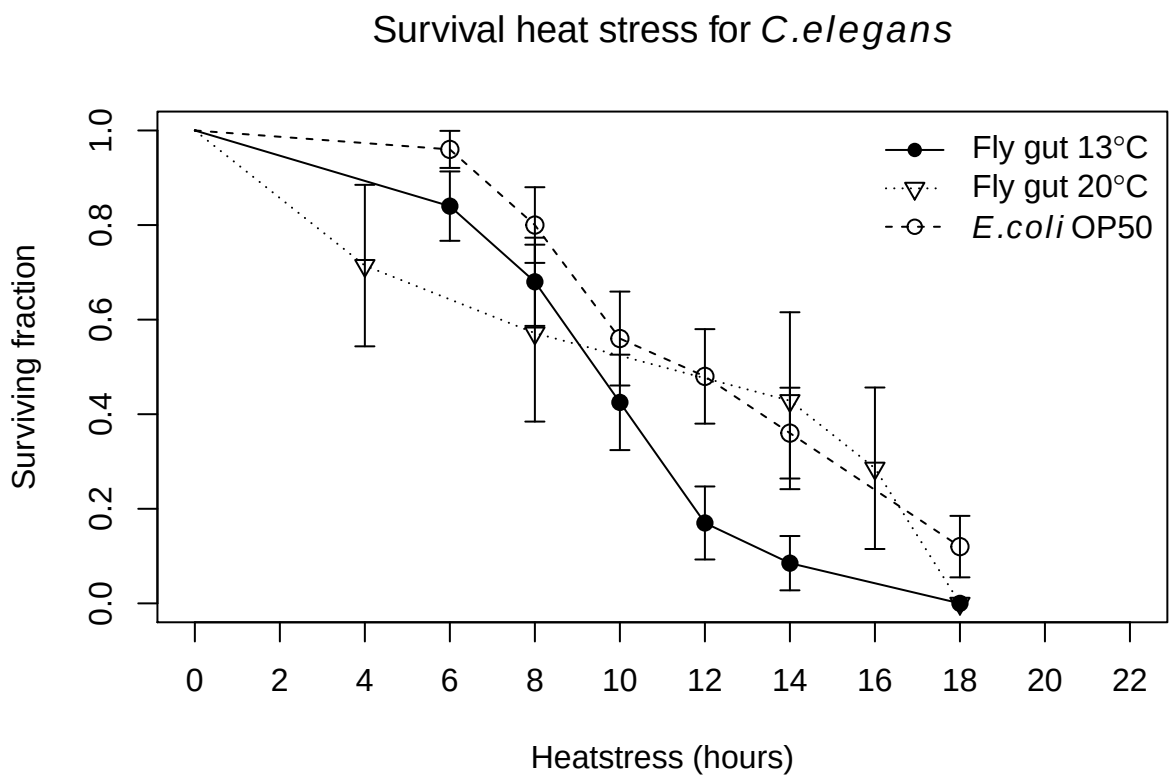
```
      df_OP50_r$Time, df_OP50_r$Surv+df_OP50_r$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50_r$Time, df_OP50_r$Surv - df_OP50_r$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
points(df_fly_20_r$Time[2:9],df_fly_20_r$Surv[2:9], pch = 6)
lines(df_fly_20_r$Time,df_fly_20_r$Surv,lty = 3)
arrows(df_fly_20_r$Time, df_fly_20_r$Surv-df_fly_20_r$Std.error, df_fly_20_r$Time,df_fly_20_r$Surv+df_fl
```

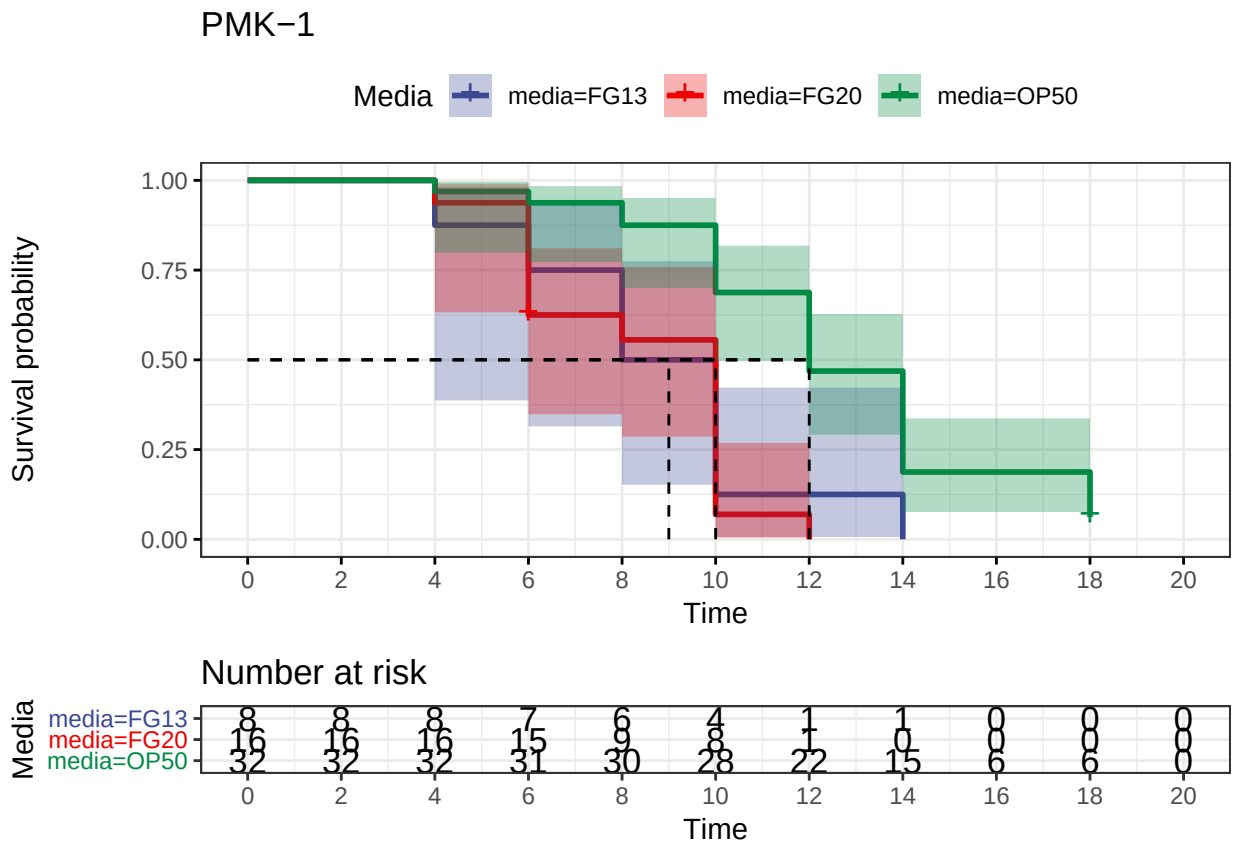
```
## Warning in arrows(df_fly_20_r$Time, df_fly_20_r$Surv -
## df_fly_20_r$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

```
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```




```
d_PMK$Survobj <- with(d_PMK,Surv(d_PMK$Time, event = d_PMK$status))
km_PMK <- survfit(Survobj ~ media, data = d_PMK,conf.type = "log-log", error = "greenwood")
s_km_PMK <- summary(km_PMK)
ggsurvplot(km_PMK,data = d_PMK, conf.int = TRUE,
```

```
          ggtheme = theme_bw(),risk.table = 0.25,
          palette ='aaas', surv.median.line = 'hv',
          legend.title = 'Media',break.x.by = 2,
          title = 'PMK-1')
```




```
df_fly_13_P <- data.frame(c(0,s_km_PMK$time[1:5]),
                          c(1,s_km_PMK$surv[1:5]),
                          c(0,s_km_PMK$std.err[1:5]))
colnames(df_fly_13_P) <- c('Time','Surv','Std.error')
df_fly_20_P <- data.frame(c(0,s_km_PMK$time[6:10]),
                          c(1,s_km_PMK$surv[6:10]),
                          c(0,s_km_PMK$std.err[6:10]))
colnames(df_fly_20_P) <- c('Time','Surv','Std.error')
df_OP50_P <- data.frame(c(0,s_km_PMK$time[11:17]),
                        c(1,s_km_PMK$surv[11:17]),
                          c(0,s_km_PMK$std.err[11:17]))
colnames(df_OP50_P) <- c('Time','Surv','Std.error')

plot(df_fly_13_P$Time[2:9],df_fly_13_P$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13_P$Time,df_fly_13_P$Surv)
arrows(df_fly_13_P$Time, df_fly_13_P$Surv-df_fly_13_P$Std.error, df_fly_13_P$Time,df_fly_13_P$Surv+df_fl
```

```
## Warning in arrows(df_fly_13_P$Time, df_fly_13_P$Surv -
## df_fly_13_P$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

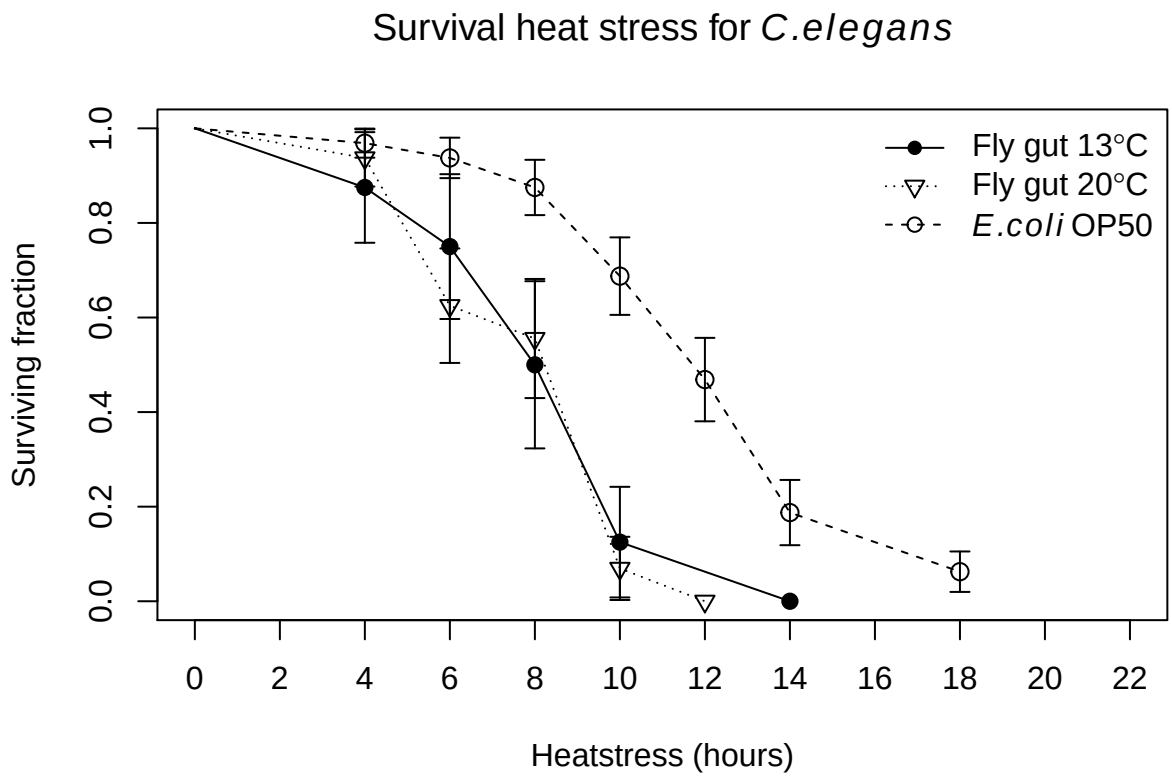
```
points(df_OP50_P$Time[2:9],df_OP50_P$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50_P$Time,df_OP50_P$Surv, lty = 2)
arrows(df_OP50_P$Time, df_OP50_P$Surv-df_OP50_P$Std.error,
      df_OP50_P$Time, df_OP50_P$Surv+df_OP50_P$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50_P$Time, df_OP50_P$Surv - df_OP50_P$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

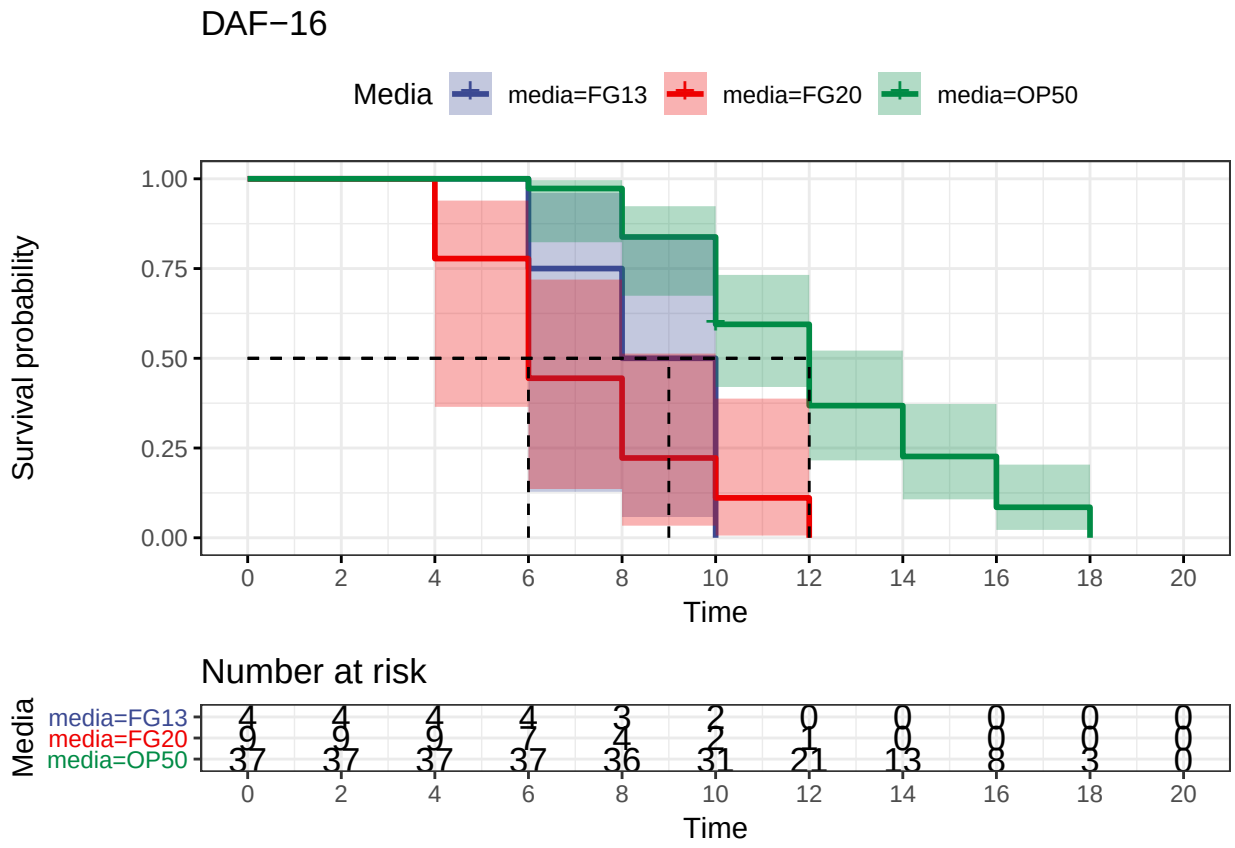
```
points(df_fly_20_P$Time[2:9],df_fly_20_P$Surv[2:9], pch = 6)
lines(df_fly_20_P$Time,df_fly_20_P$Surv,lty = 3)
arrows(df_fly_20_P$Time, df_fly_20_P$Surv-df_fly_20_P$Std.error, df_fly_20_P$Time,df_fly_20_P$Surv+df_fl
```

```
## Warning in arrows(df_fly_20_P$Time, df_fly_20_P$Surv -
## df_fly_20_P$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

```
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```



```
d_DAF$Survobj <- with(d_DAF,Surv(d_DAF$Time, event = d_DAF$status))
km_DAF <- survfit(Survobj ~ media, data = d_DAF,conf.type = "log-log", error = "greenwood")
s_km_DAF <- summary(km_DAF)
ggsurvplot(km_DAF,data = d_DAF, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'DAF-16')
```




```
df_fly_13_d <- data.frame(c(0,s_km_DAF$time[1:3]),
                          c(1,s_km_DAF$surv[1:3]),
                          c(0,s_km_DAF$std.err[1:3]))
colnames(df_fly_13_d) <- c('Time','Surv','Std.error')
df_fly_20_d <- data.frame(c(0,s_km_DAF$time[4:8]),
                          c(1,s_km_DAF$surv[4:8]),
                          c(0,s_km_DAF$std.err[4:8]))
colnames(df_fly_20_d) <- c('Time','Surv','Std.error')
df_OP50_d <- data.frame(c(0,s_km_DAF$time[9:15]),
                        c(1,s_km_DAF$surv[9:15]),
                          c(0,s_km_DAF$std.err[9:15]))
colnames(df_OP50_d) <- c('Time','Surv','Std.error')

plot(df_fly_13_d$Time[2:9],df_fly_13_d$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
```

```
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13_d$Time,df_fly_13_d$Surv)
arrows(df_fly_13_d$Time, df_fly_13_d$Surv-df_fly_13_d$Std.error, df_fly_13_d$Time,df_fly_13_d$Surv+df_fl
```

```
## Warning in arrows(df_fly_13_d$Time, df_fly_13_d$Surv -
## df_fly_13_d$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

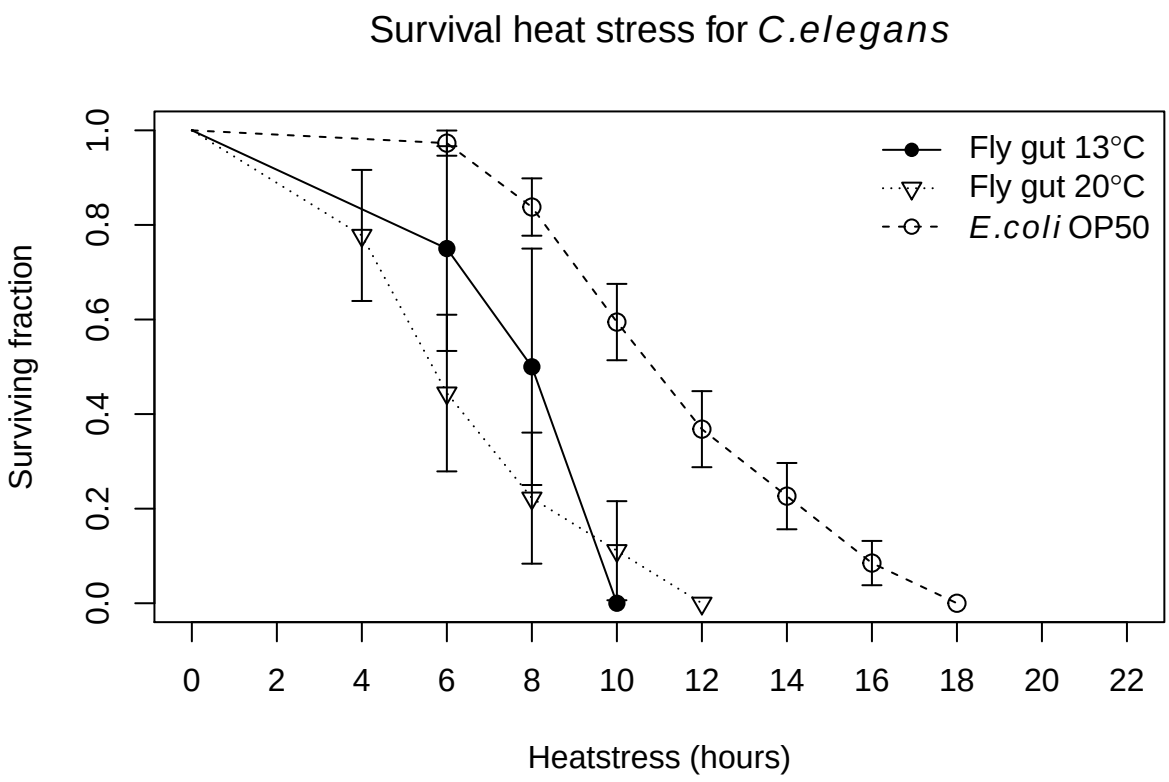
```
points(df_OP50_d$Time[2:9],df_OP50_d$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50_d$Time,df_OP50_d$Surv, lty = 2)
arrows(df_OP50_d$Time, df_OP50_d$Surv-df_OP50_d$Std.error,
      df_OP50_d$Time, df_OP50_d$Surv+df_OP50_d$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50_d$Time, df_OP50_d$Surv - df_OP50_d$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
points(df_fly_20_d$Time[2:9],df_fly_20_d$Surv[2:9], pch = 6)
lines(df_fly_20_d$Time,df_fly_20_d$Surv,lty = 3)
arrows(df_fly_20_d$Time, df_fly_20_d$Surv-df_fly_20_d$Std.error, df_fly_20_d$Time,df_fly_20_d$Surv+df_fl
```

```
## Warning in arrows(df_fly_20_d$Time, df_fly_20_d$Surv -
## df_fly_20_d$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
```

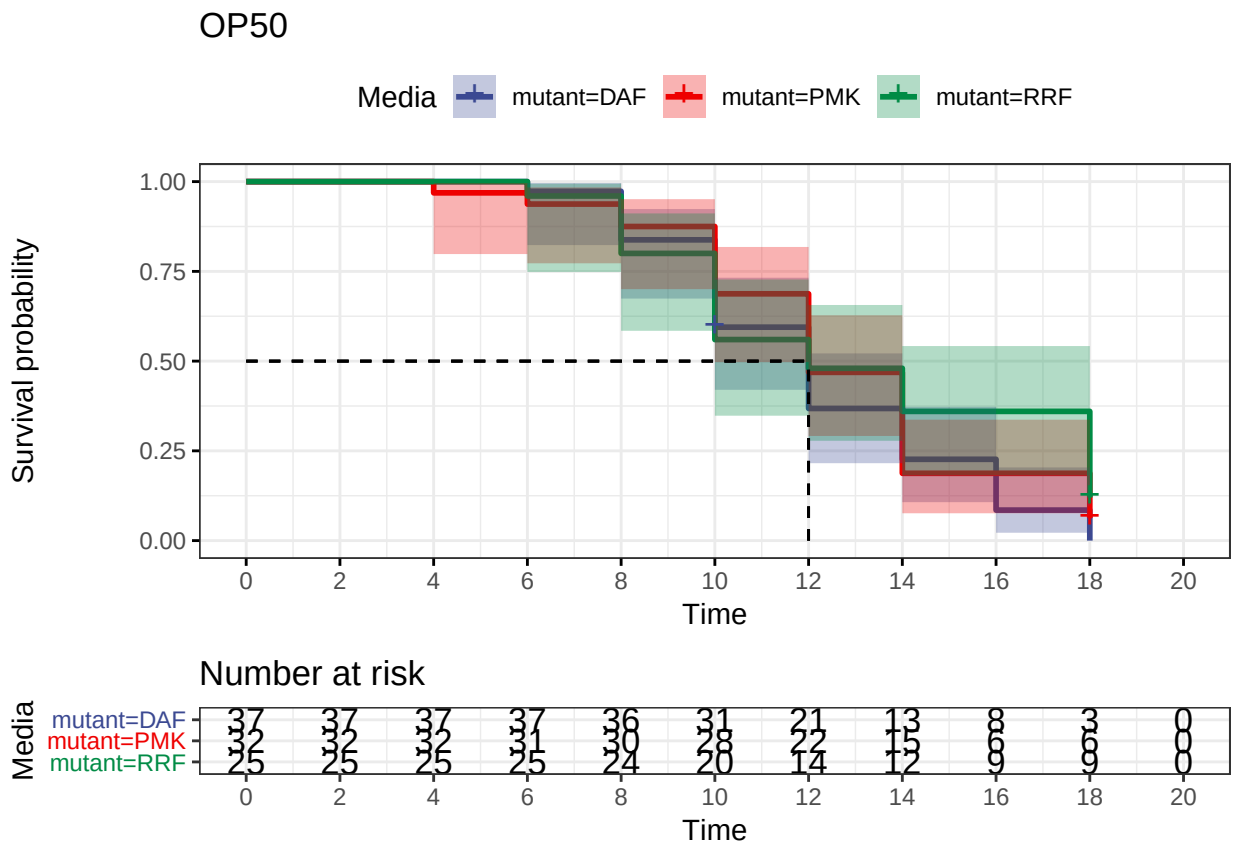
```
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```



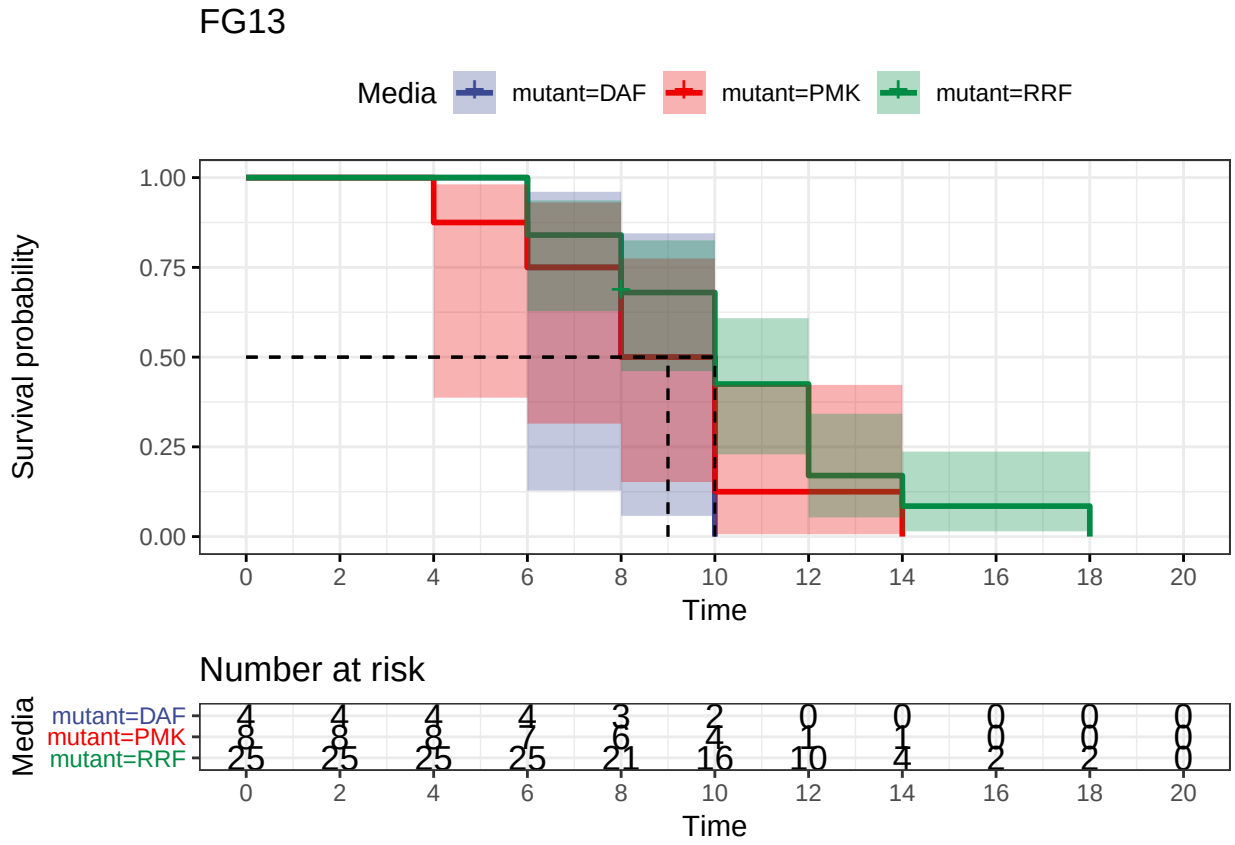
Then we compare by medium

```
data_OP50 <- subset(data, media == 'OP50')
data_FG13 <- subset(data, media == 'FG13')
data_FG20 <- subset(data, media == 'FG20')
```

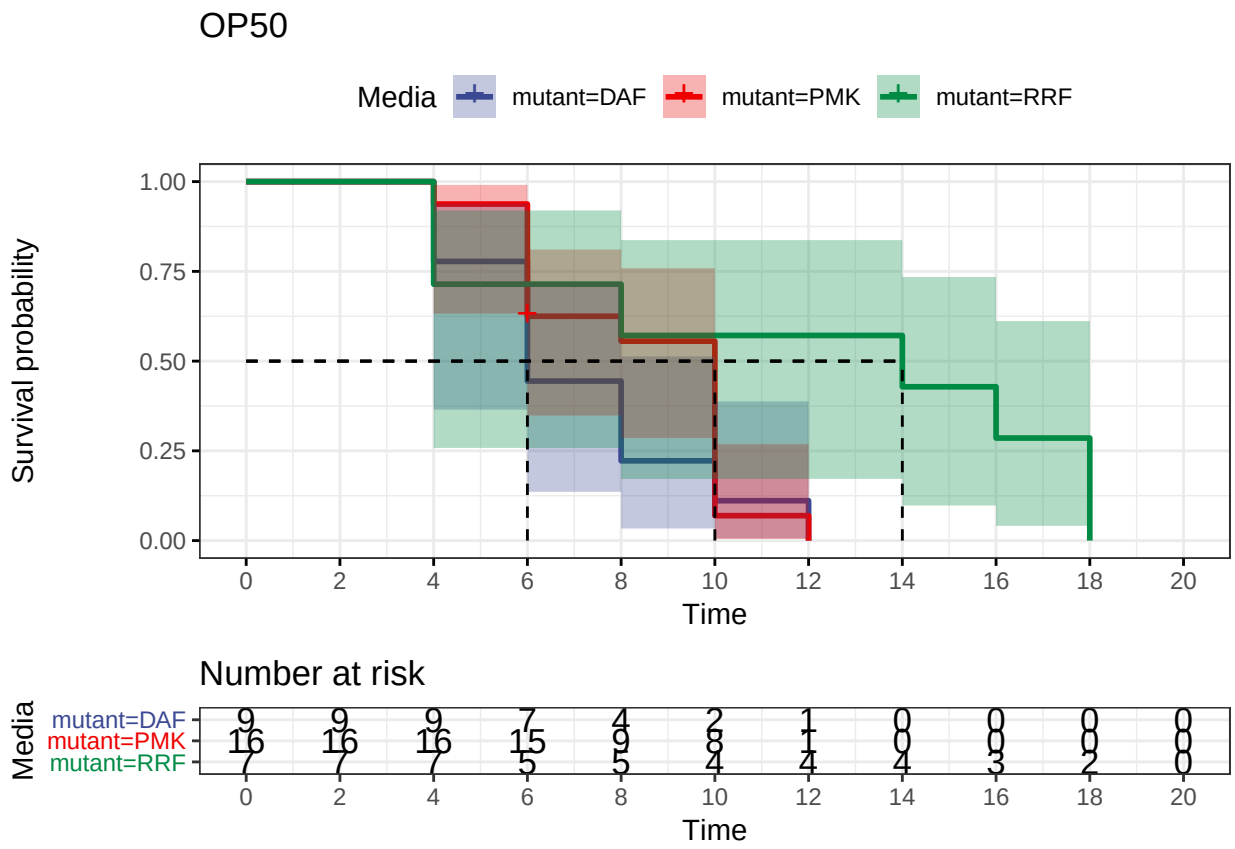
```
data_OP50$Survobj <- with(data_OP50,Surv(data_OP50$Time, event = data_OP50$status))
km_OP50 <- survfit(Survobj ~ mutant, data = data_OP50,conf.type = "log-log", error = "greenwood")
s_km_OP50 <- summary(km_OP50)
ggsurvplot(km_OP50,data = data_OP50, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'OP50')
```




```
data_FG13$Survobj <- with(data_FG13,Surv(data_FG13$Time, event = data_FG13$status))
km_FG13 <- survfit(Survobj ~ mutant, data = data_FG13,conf.type = "log-log", error = "greenwood")
s_km_FG13 <- summary(km_FG13)
ggsurvplot(km_FG13,data = data_FG13, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'FG13')
```




```
data_FG20$Survobj <- with(data_FG20,Surv(data_FG20$Time, event = data_FG20$status))
km_FG20 <- survfit(Survobj ~ mutant, data = data_FG20,conf.type = "log-log", error = "greenwood")
s_km_FG20 <- summary(km_FG20)
ggsurvplot(km_FG20,data = data_FG20, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'OP50')
```



Shows errorbars and lineplot based on one of Anders papers https://onlinelibrary.wiley.com/doi/full/10.1111/acel.12165 This is done by extracting the surviving proportion, standard errors and time from survfit(). and adding a startpoint where the survival is 100% at 0 hours.

Now we test for difference between the curves with both log-rank and gehan-wilcoxon and with an cox proportional hazard model

```
data$Survobj <- with(data,Surv(data$Time, event = data$status))
survdiff(Survobj ~media + mutant, data = data, rho = 0)
```

```
## Call:
## survdiff(formula = Survobj ~ media + mutant, data = data, rho = 0)
##
##                           N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13, mutant=DAF  4        4     1.78     2.762     3.545
## media=FG13, mutant=PMK  8        8     4.22     3.384     4.615
## media=FG13, mutant=RRF 25       24    19.78     0.901     1.483
## media=FG20, mutant=DAF  9        9     2.82    13.539    17.336
## media=FG20, mutant=PMK 16       15     6.88     9.595    13.238
## media=FG20, mutant=RRF  7        7     8.29     0.200     0.341
## media=OP50, mutant=DAF 37       36    38.84     0.208     0.404
## media=OP50, mutant=PMK 32       30    39.06     2.100     4.263
## media=OP50, mutant=RRF 25       22    33.34     3.855     7.900
##
##  Chisq= 51.7  on 8 degrees of freedom, p= 2e-08
```

```r
survdiff(Survobj ~media + mutant, data = data, rho = 1)
```

```
## Call:
## survdiff(formula = Survobj ~ media + mutant, data = data, rho = 1)
##
##                          N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13, mutant=DAF   4     3.24     1.50    2.0432    2.9683
## media=FG13, mutant=PMK   8     6.12     3.18    2.7304    4.3317
## media=FG13, mutant=RRF  25    15.24    12.93    0.4151    0.8063
## media=FG20, mutant=DAF   9     7.76     2.32   12.7917   18.3933
## media=FG20, mutant=PMK  16    12.12     5.63    7.4975   11.8513
## media=FG20, mutant=RRF   7     3.62     3.99    0.0348    0.0719
## media=OP50, mutant=DAF  37    18.26    23.74    1.2635    2.9998
## media=OP50, mutant=PMK  32    14.58    22.00    2.5059    6.0333
## media=OP50, mutant=RRF  25    11.37    17.04    1.8896    4.3076
##
##  Chisq= 48  on 8 degrees of freedom, p= 1e-07
```

```r
fit_both<- coxph(Survobj ~media + mutant, data = data)
summary(fit_both)
```

```
## Call:
## coxph(formula = Survobj ~ media + mutant, data = data)
##
##   n= 163, number of events= 155
##
##                coef exp(coef)  se(coef)      z Pr(>|z|)
## mediaFG20  0.005922  1.005940  0.250871  0.024 0.981166
## mediaOP50 -1.147199  0.317525  0.231637 -4.953 7.32e-07 ***
## mutantPMK -0.253909  0.775762  0.202013 -1.257 0.208791
## mutantRRF -0.870655  0.418677  0.229717 -3.790 0.000151 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20    1.0059     0.9941    0.6152    1.6448
## mediaOP50    0.3175     3.1494    0.2017    0.5000
## mutantPMK    0.7758     1.2891    0.5221    1.1526
## mutantRRF    0.4187     2.3885    0.2669    0.6568
##
## Concordance= 0.67  (se = 0.028 )
## Rsquare= 0.211   (max possible= 1 )
## Likelihood ratio test= 38.69  on 4 df,   p=8e-08
## Wald test            = 38.5  on 4 df,   p=9e-08
## Score (logrank) test = 39.91  on 4 df,   p=5e-08
```

```r
AIC(fit_both)
```

```
## [1] 1273.218
```

```r
fit_int <- coxph(Survobj ~media + mutant + mutant:media, data = data)
AIC(fit_int)
```

```
## [1] 1274.41
```

```r
fit_med <- coxph(Survobj ~media, data = data)
summary(fit_med)
```

```
## Call:
## coxph(formula = Survobj ~ media, data = data)
##
##   n= 163, number of events= 155
##
##              coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20  0.1971    1.2178   0.2456  0.802 0.422369
## mediaOP50 -0.7195    0.4870   0.2019 -3.564 0.000365 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20     1.218     0.8211    0.7525    1.9709
## mediaOP50     0.487     2.0535    0.3278    0.7234
##
## Concordance= 0.649  (se = 0.025 )
## Rsquare= 0.131   (max possible= 1 )
## Likelihood ratio test= 22.98  on 2 df,   p=1e-05
## Wald test            = 24.52  on 2 df,   p=5e-06
## Score (logrank) test = 25.84  on 2 df,   p=2e-06
```

```r
AIC(fit_med)
```

```
## [1] 1284.932
```

```r
fit_mut <- coxph(Survobj ~mutant, data = data)
summary(fit_mut)
```

```
## Call:
## coxph(formula = Survobj ~ mutant, data = data)
##
##   n= 163, number of events= 155
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## mutantPMK -0.08361   0.91979  0.19910 -0.420    0.675
## mutantRRF -0.33095   0.71824  0.20139 -1.643    0.100
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mutantPMK    0.9198      1.087    0.6226     1.359
## mutantRRF    0.7182      1.392    0.4840     1.066
##
## Concordance= 0.526  (se = 0.029 )
## Rsquare= 0.018   (max possible= 1 )
```

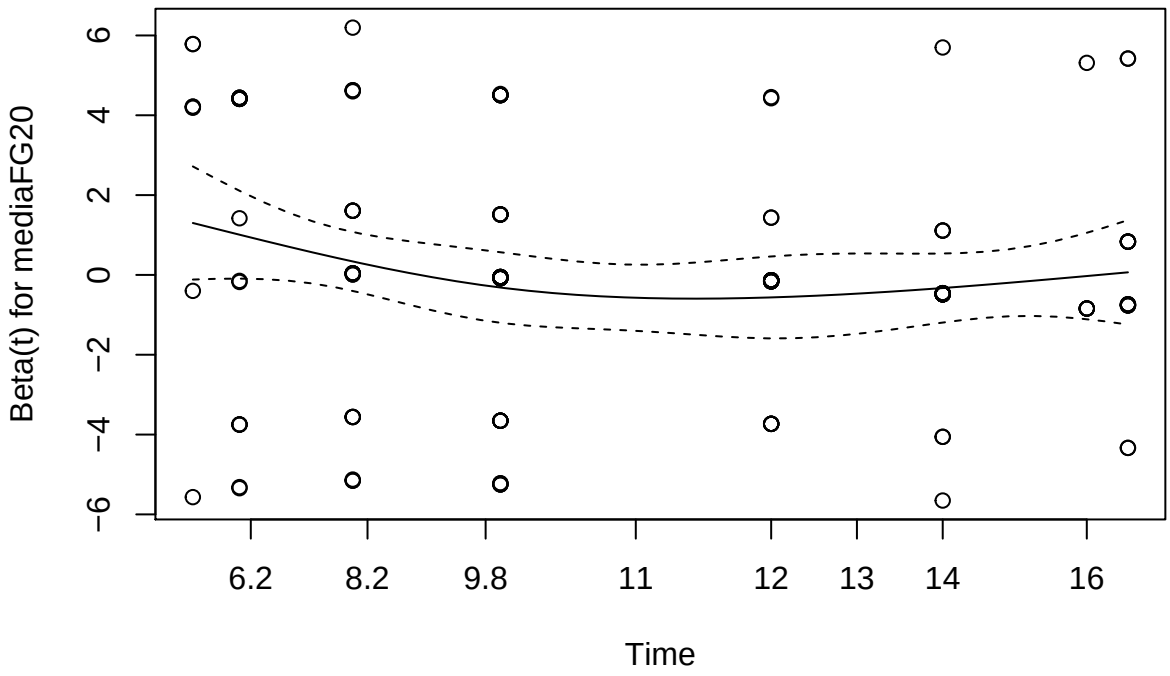
```
## Likelihood ratio test= 3.03  on 2 df,   p=0.2
## Wald test            = 2.97  on 2 df,   p=0.2
## Score (logrank) test = 2.99  on 2 df,   p=0.2
```

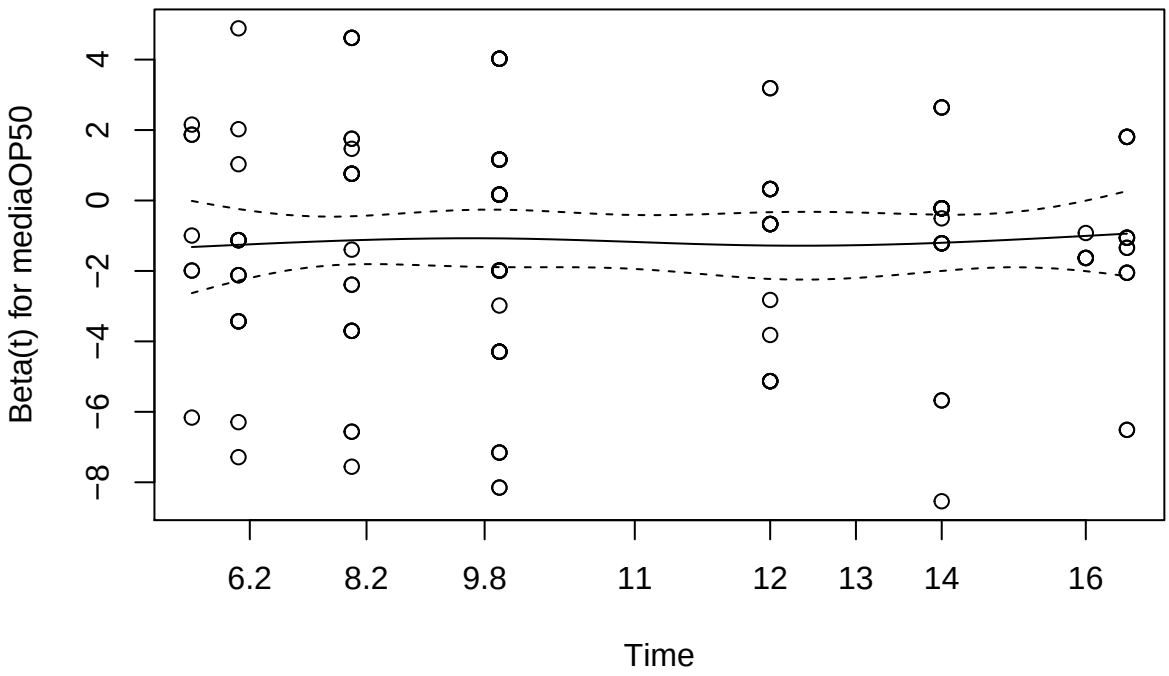
```
AIC(fit_mut)
```

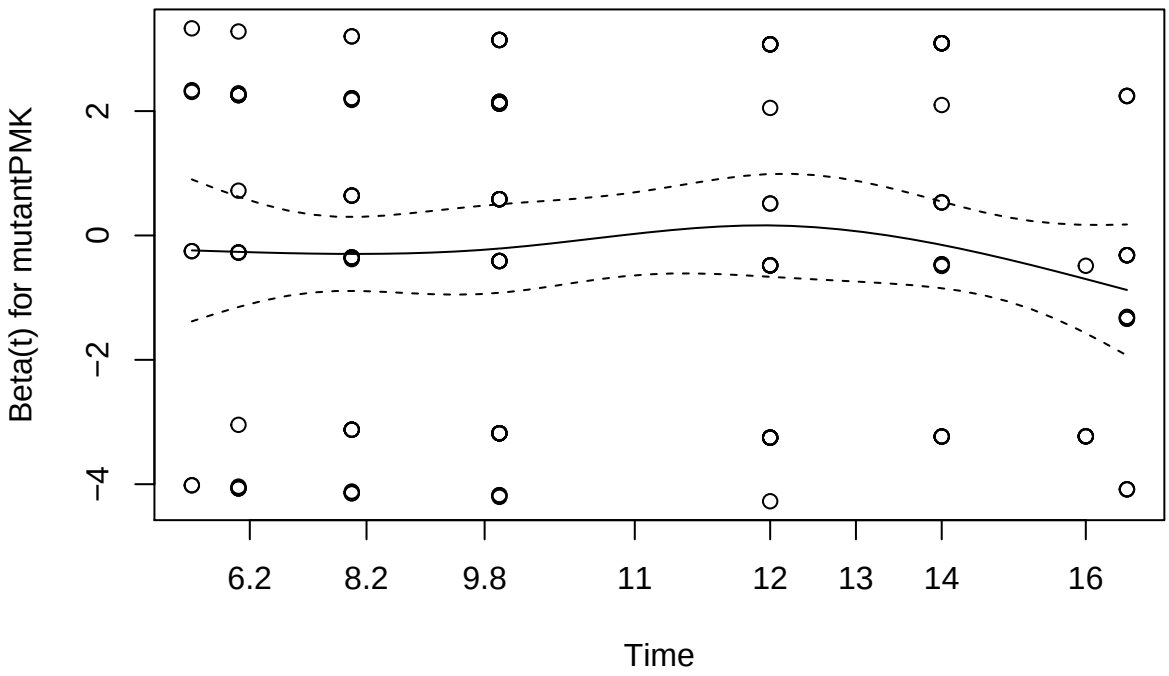
```
## [1] 1304.882
```

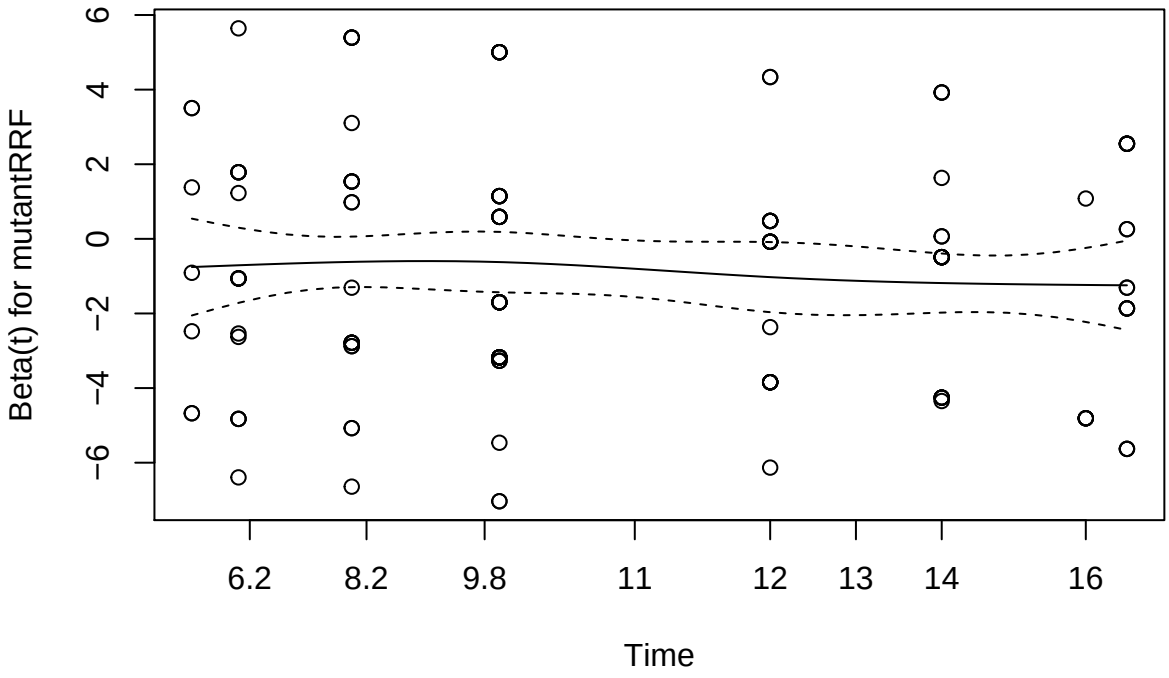
Best model must include both media and mutant strain but not necessarily an interaction between them. This could be driven by few observations for FG20 in RRF-3 Then we do a check on the proportional hazard assumption

```
prop_both <- cox.zph(fit_both)
prop_int <- cox.zph(fit_int)
plot(prop_both)
```

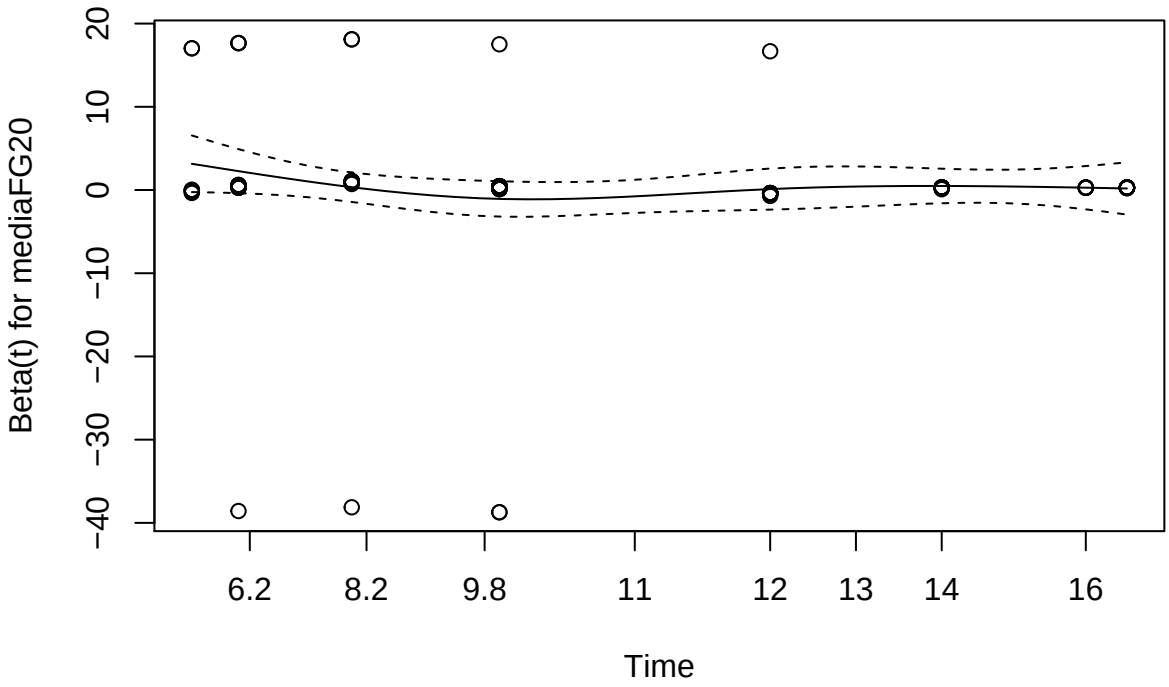


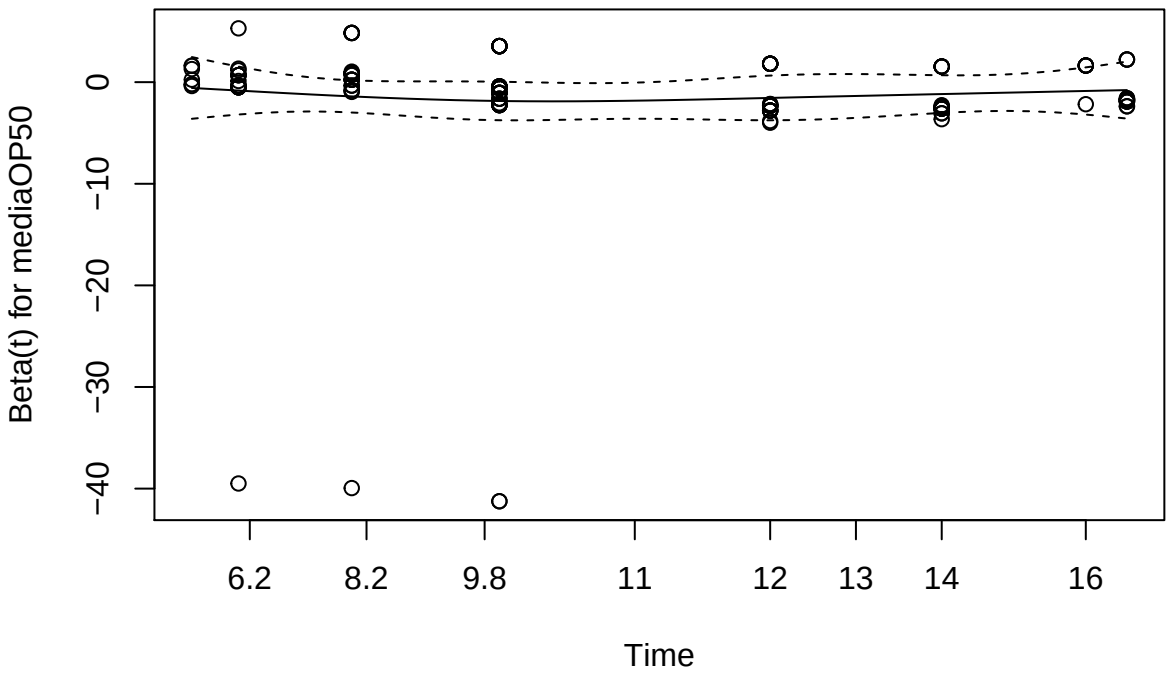

Beta(t) for mediaOP50
4
2
0
−2
−4
−6
−8
6.2
8.2
9.8
11
12
13
14
16
Time

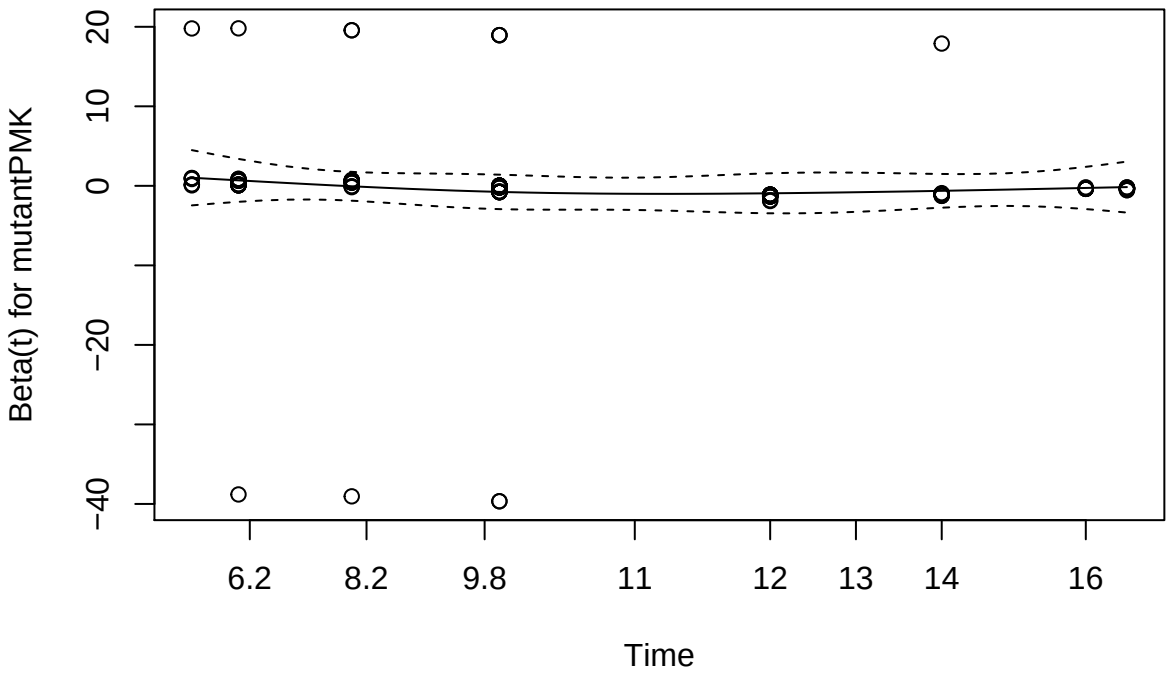

Beta(t) for mutantPMK
2
0
−2
−4
6.2
8.2
9.8
11
12
13
14
16
Time

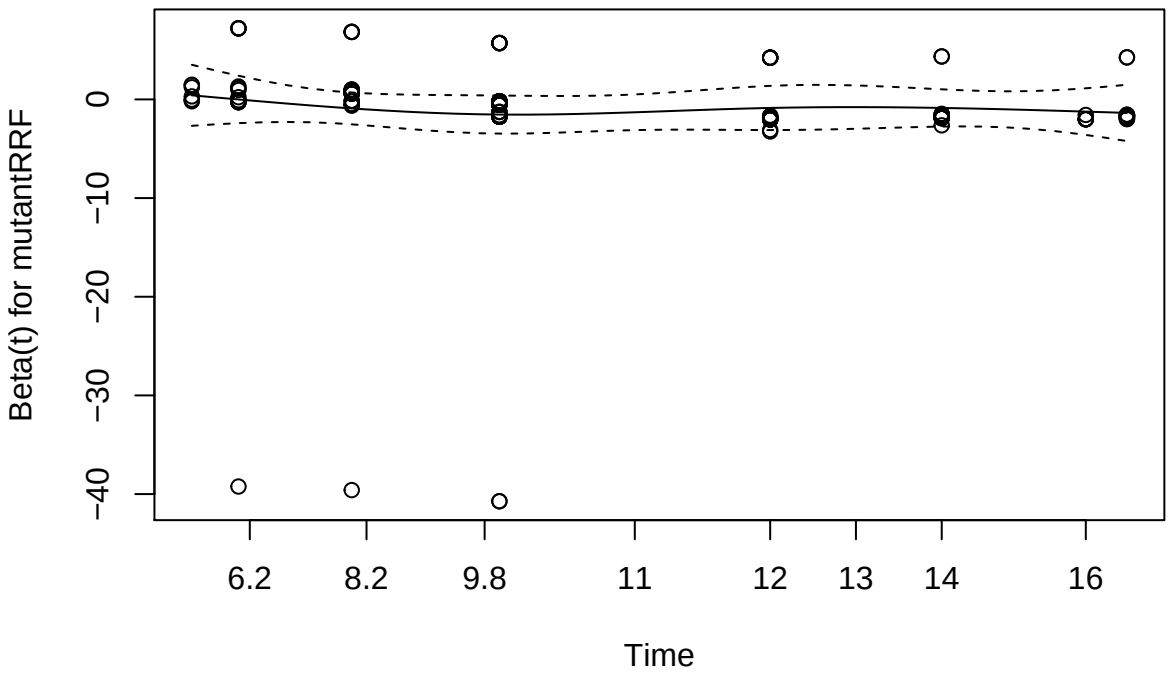


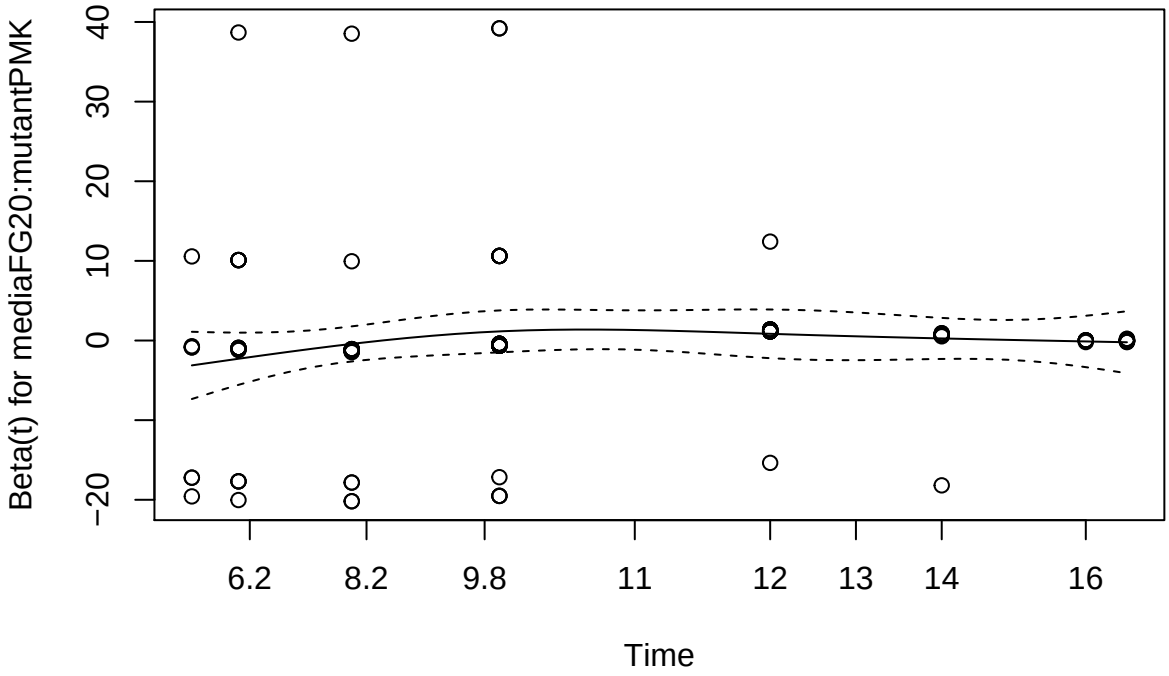

```
plot(prop_int)
```

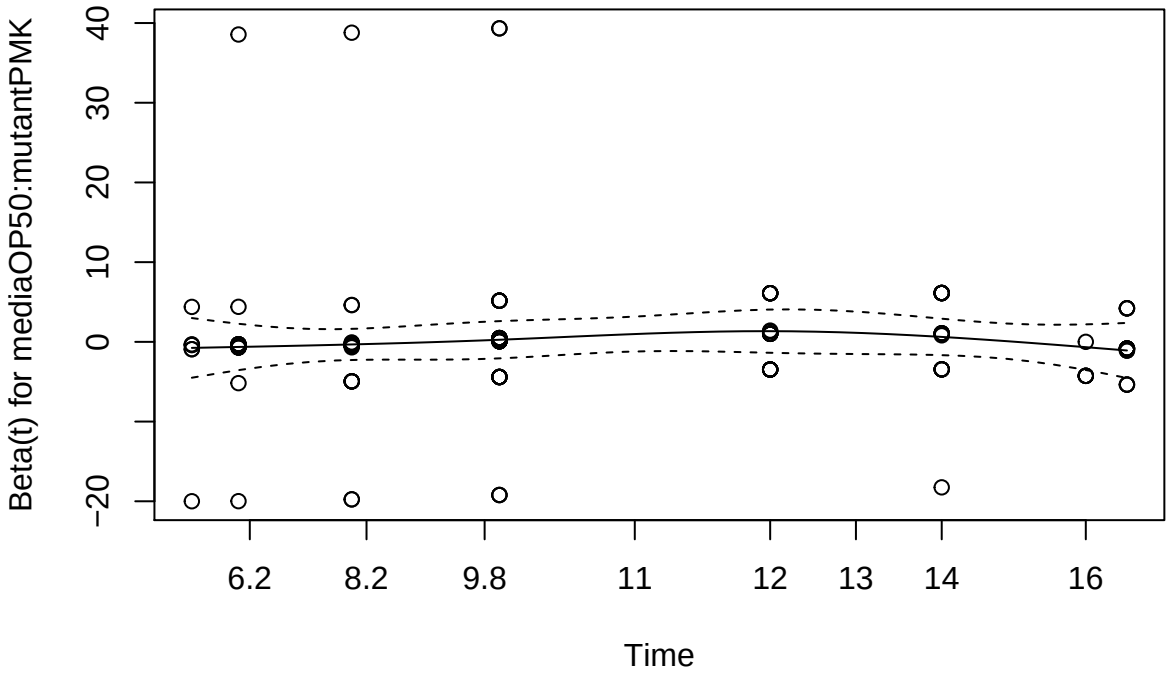

Beta(t) for mediaFG20
20
10
0
−10
−20
−30
−40
6.2
8.2
9.8
11
12
13
14
16
Time

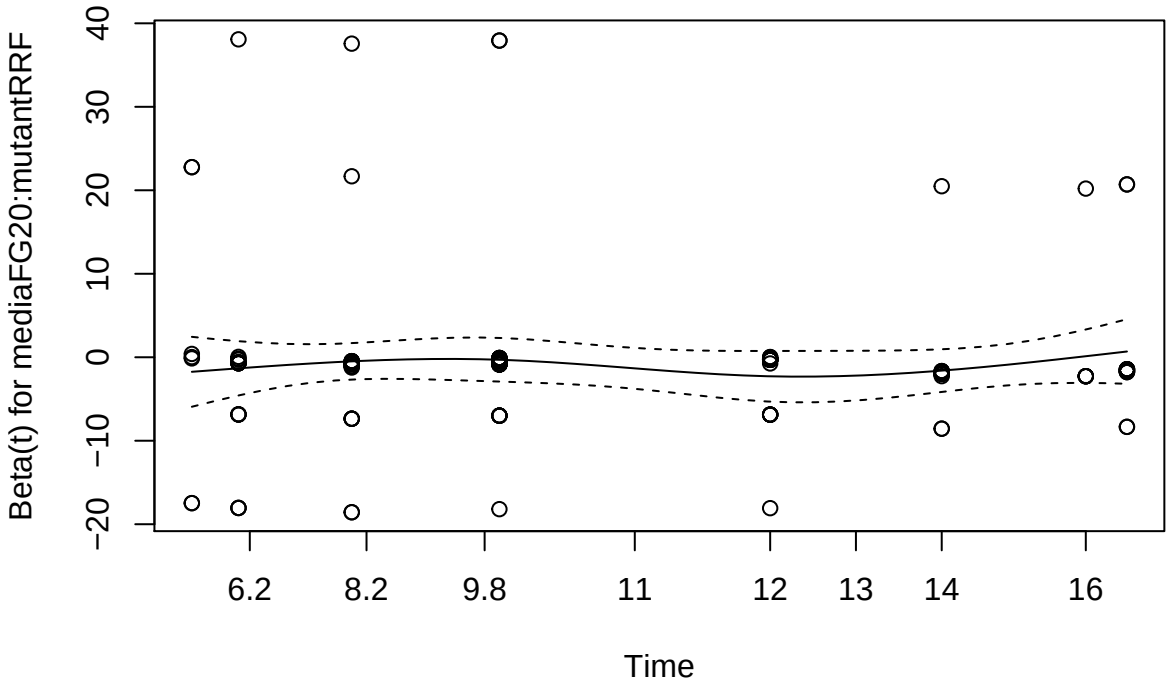

Beta(t) for mediaOP50
0
−10
−20
−30
−40
6.2
8.2
9.8
11
12
13
14
16
Time

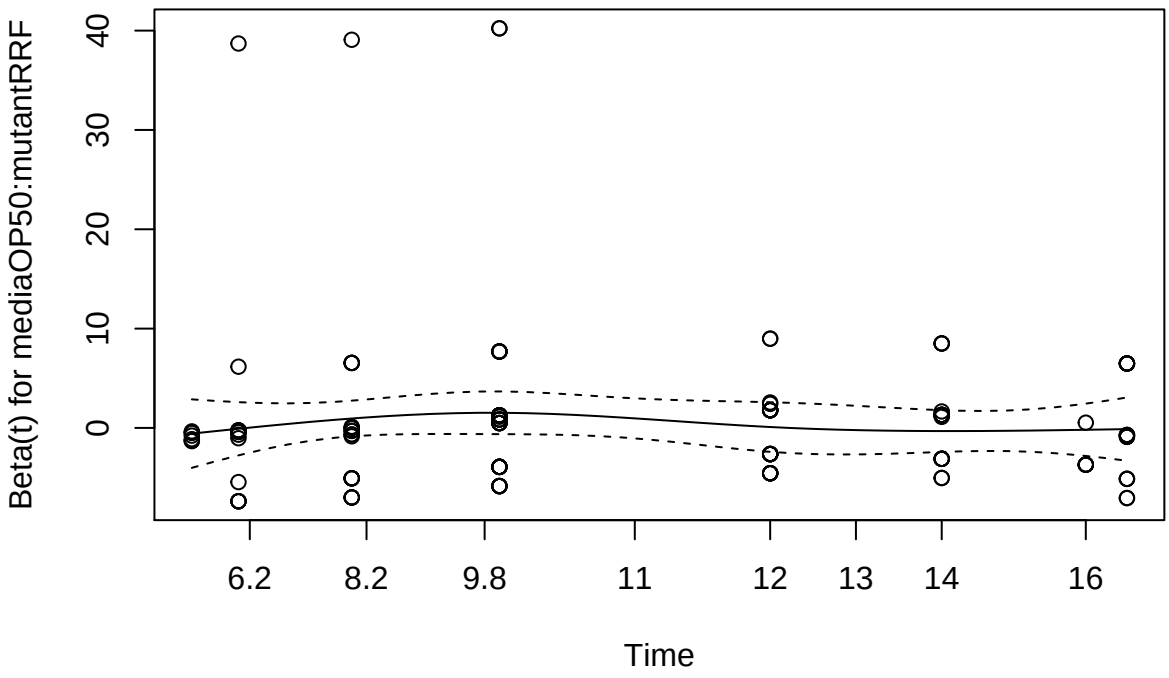

Beta(t) for mutantPMK
20
10
0
−20
−40
6.2
8.2
9.8
11
12
13
14
16
Time


Beta(t) for mutantRRF
0
−10
−20
−30
−40
6.2
8.2
9.8
11
12
13
14
16
Time


Beta(t) for mediaFG20:mutantPMK
40
30
20
10
0
−20
6.2
8.2
9.8
11
12
13
14
16
Time


Beta(t) for mediaOP50:mutantPMK
40
30
20
10
0
−20
6.2
8.2
9.8
11
12
13
14
16
Time

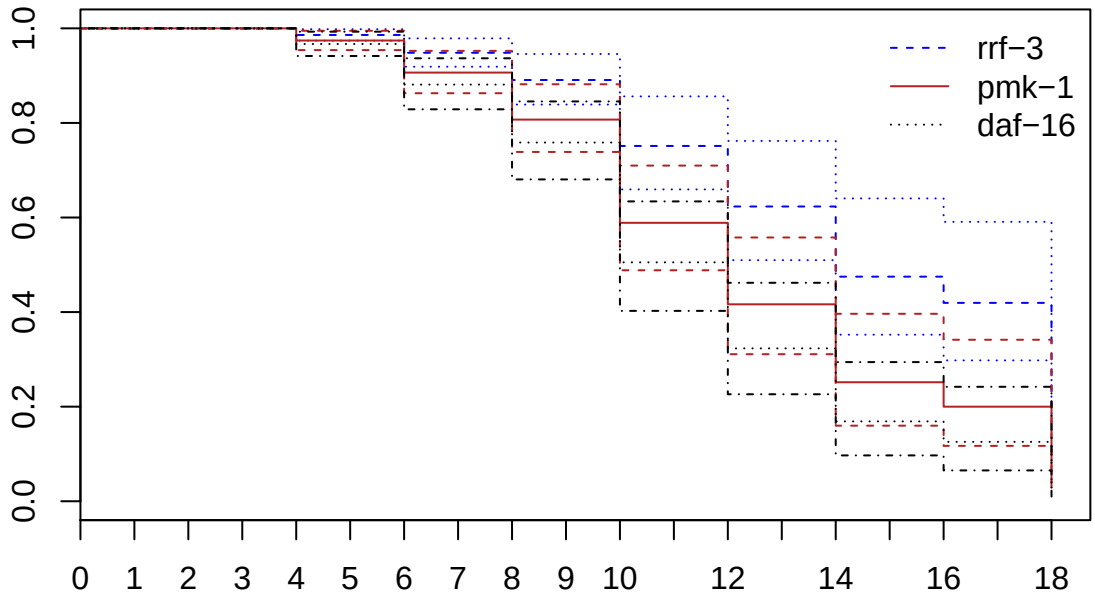

Beta(t) for mediaFG20:mutantRRF
40
30
20
10
0
−10
−20
6.2
8.2
9.8
11
12
13
14
16
Time



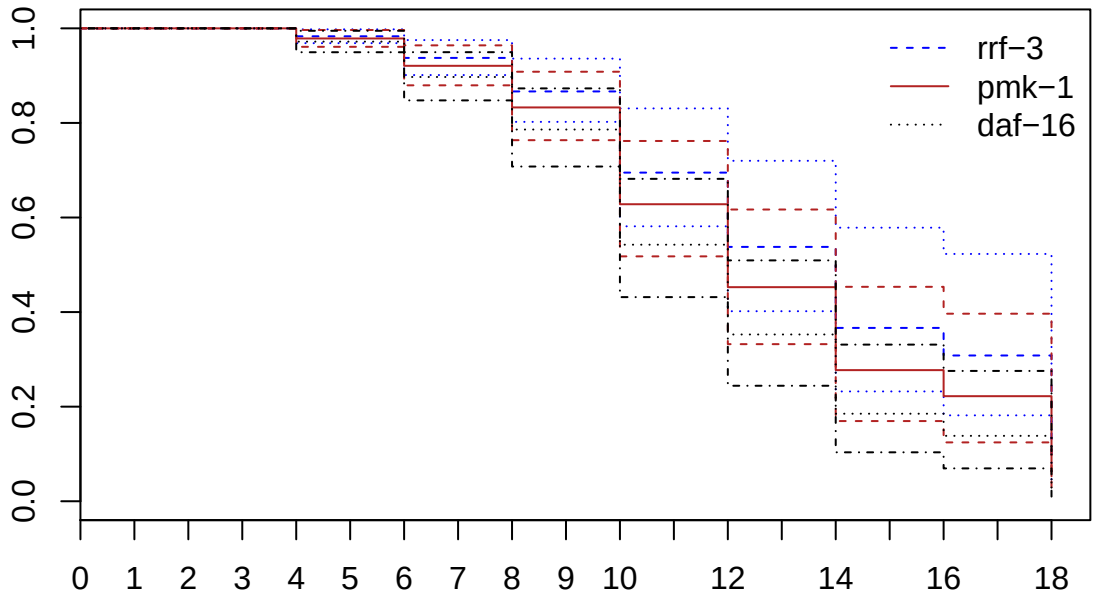
Both schoenfield residuals and the test is not significant so we proceed as if the assumptions is true for our dataset.

Then we plot predictions for both models

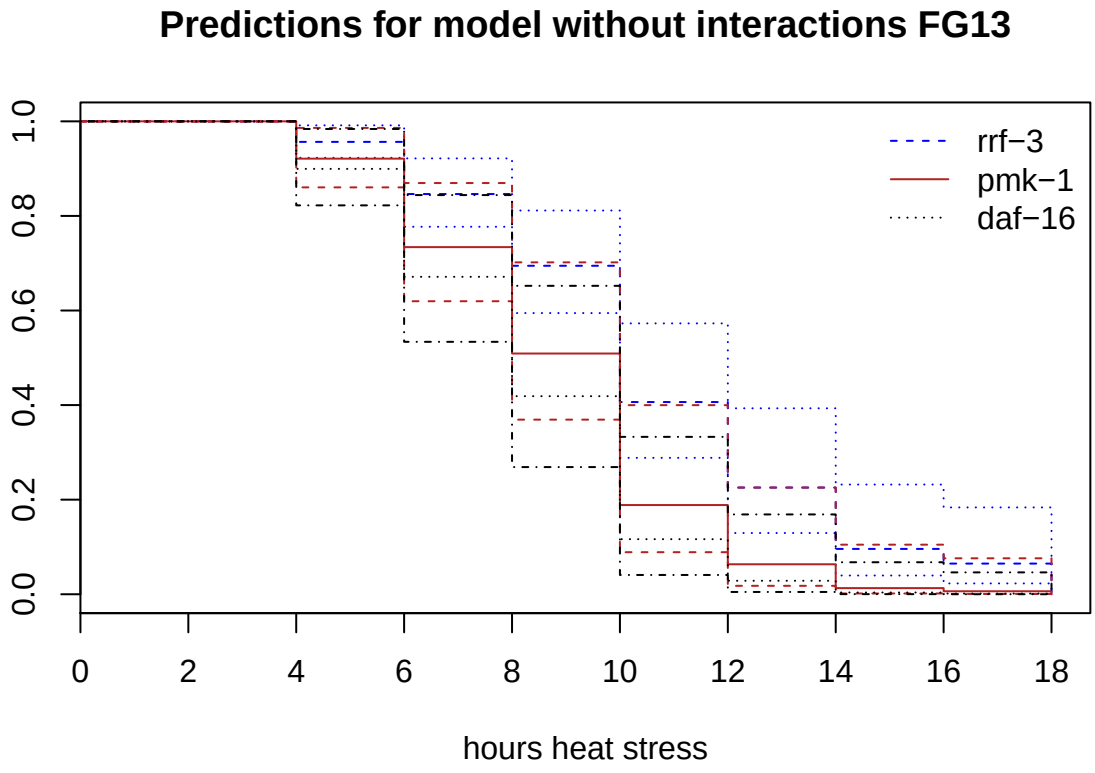
```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'OP50')), lty = 2, xaxp = c(0,18,18
     main = 'Predictions for model without interactions OP50')
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'OP50')), col = 'firebrick')
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'OP50')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n')
```



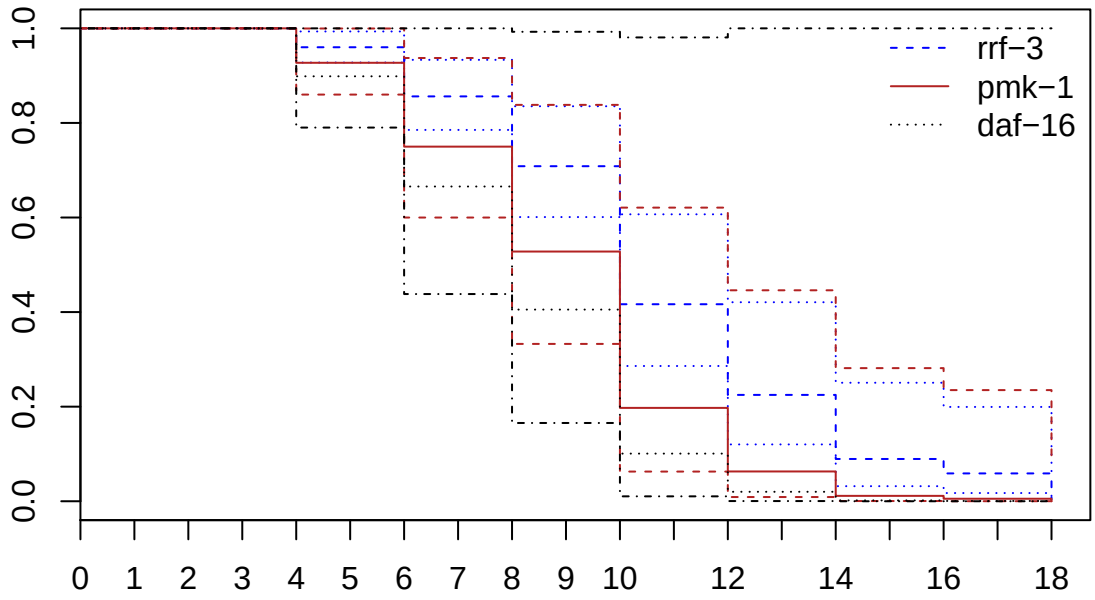

```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'OP50')), lty = 2, xaxp = c(0,18,18)
     main = 'Predictions for model with interactions OP50')
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'OP50')), col = 'firebrick')
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'OP50')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n')
```



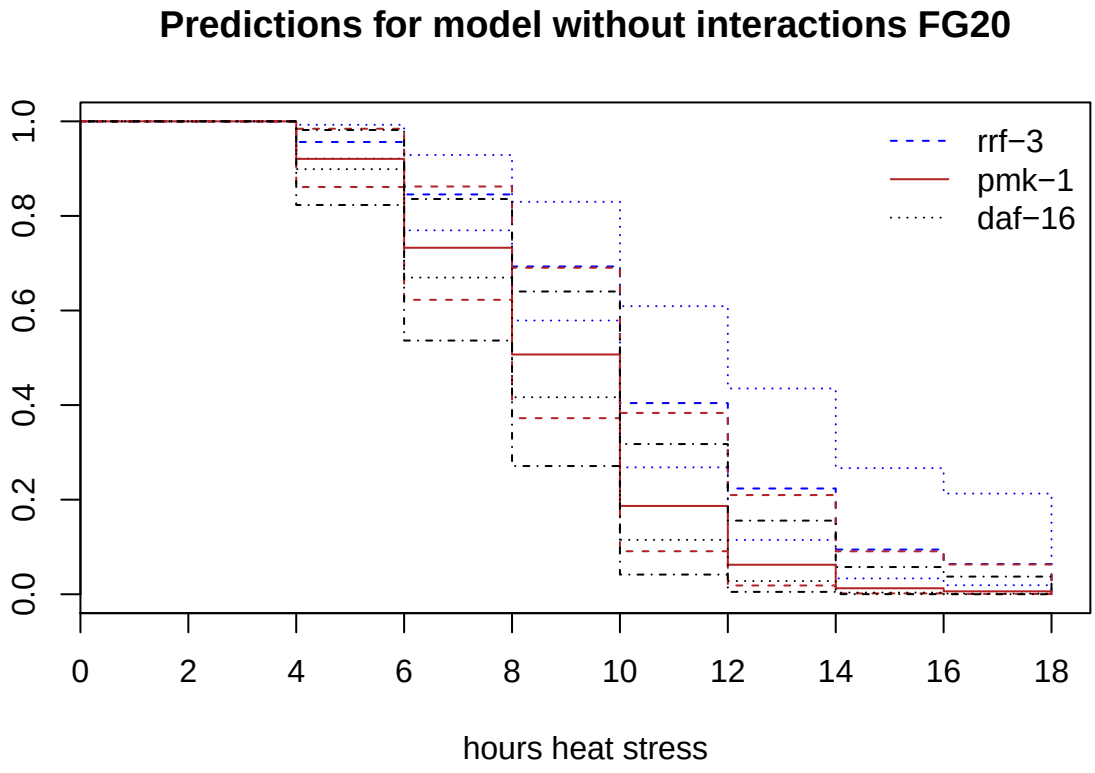

```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'FG13')), lty = 2, xaxp = c(0,18,9)
     main = 'Predictions for model without interactions FG13',
     xlab = 'hours heat stress')
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'FG13')), col = 'firebrick')
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'FG13')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
        lty = c(2,1,3), col = c('blue','firebrick','black'),
        bty = 'n')
```



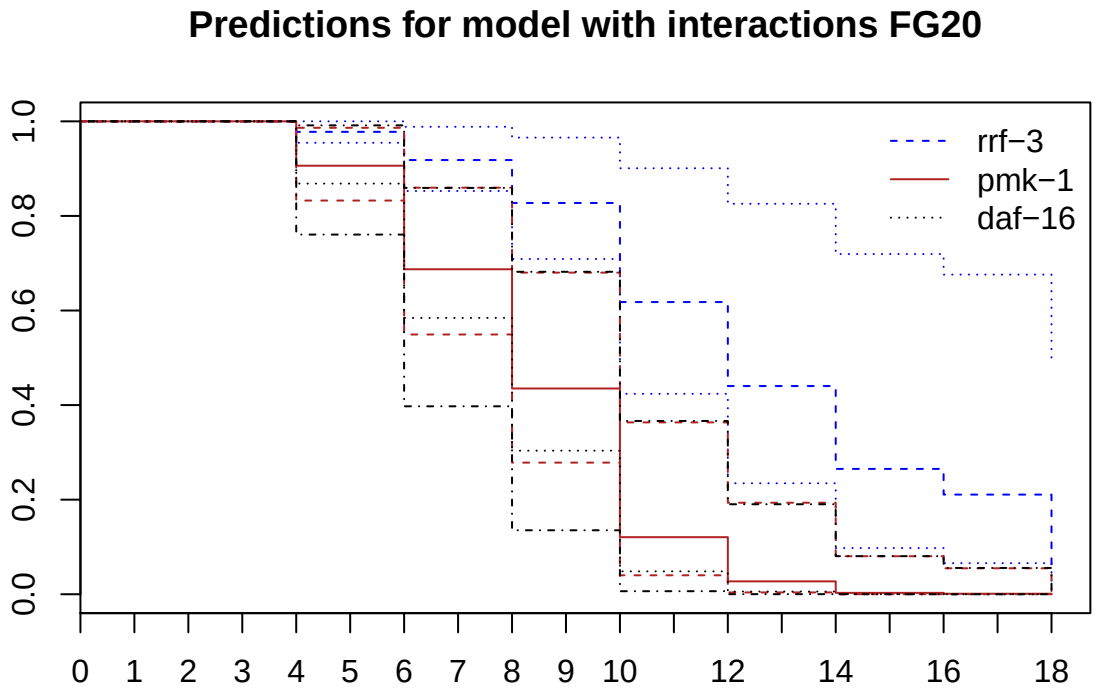

```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'FG13')), lty = 2, xaxp = c(0,18,18)
     main = 'Predictions for model with interactions FG13')
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'FG13')), col = 'firebrick')
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'FG13')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n')
```




```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'FG20')), lty = 2, xaxp = c(0,18,9)
     main = 'Predictions for model without interactions FG20',
     xlab = 'hours heat stress')
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'FG20')), col = 'firebrick')
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'FG20')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
        lty = c(2,1,3), col = c('blue','firebrick','black'),
        bty = 'n')
```

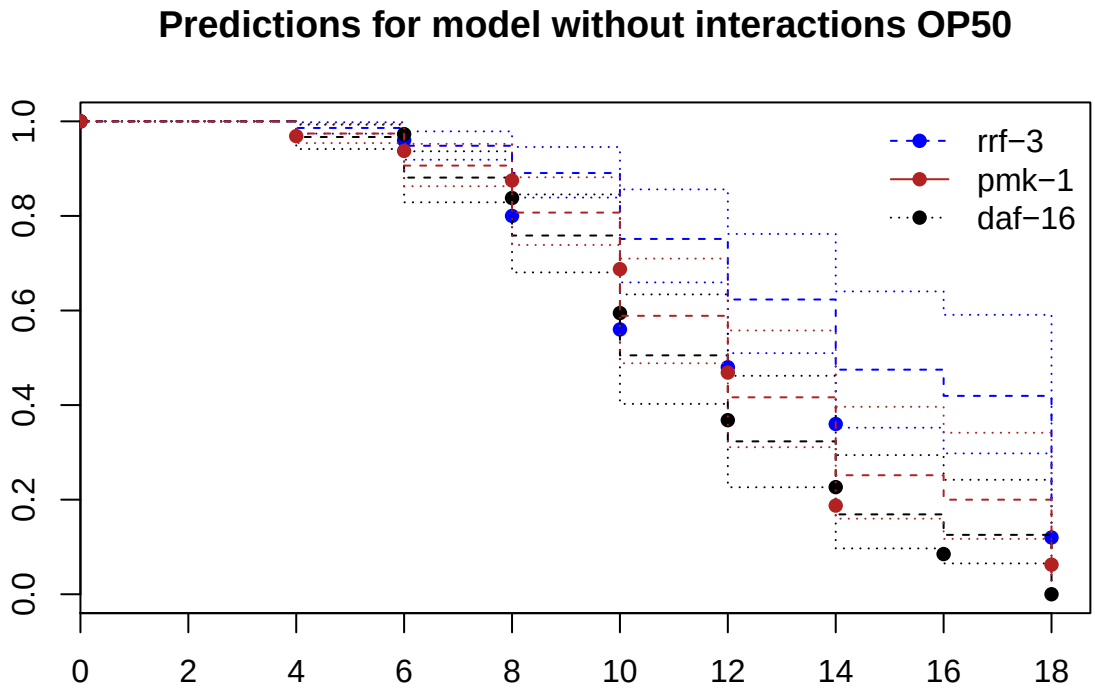



```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'FG20')), lty = 2, xaxp = c(0,18,18)
     main = 'Predictions for model with interactions FG20')
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'FG20')), col = 'firebrick')
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'FG20')), lty =3)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n')
```



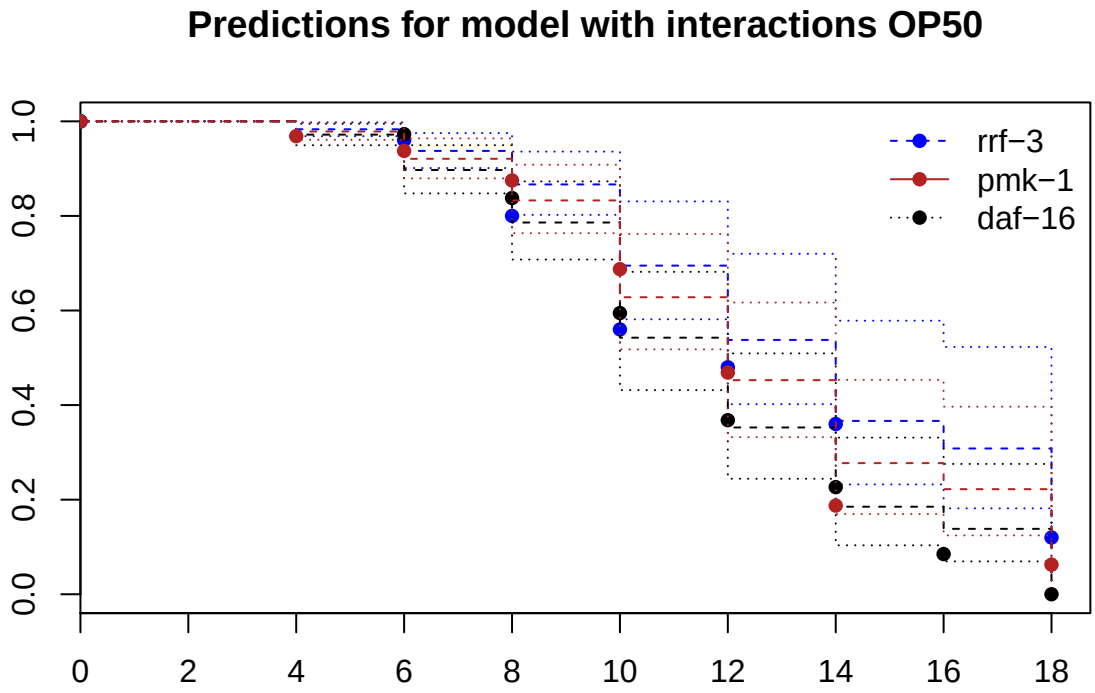
We check how well predictions line up with reality

```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'OP50')), lty = 2, xaxp = c(0,18,9)
     main = 'Predictions for model without interactions OP50')
points(df_OP50_r$Time,df_OP50_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'OP50')), lty = 2, col = 'black',)
points(df_OP50_d$Time,df_OP50_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'OP50')), lty = 2, col = 'firebric
points(df_OP50_P$Time,df_OP50_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n', pch = 16)
```



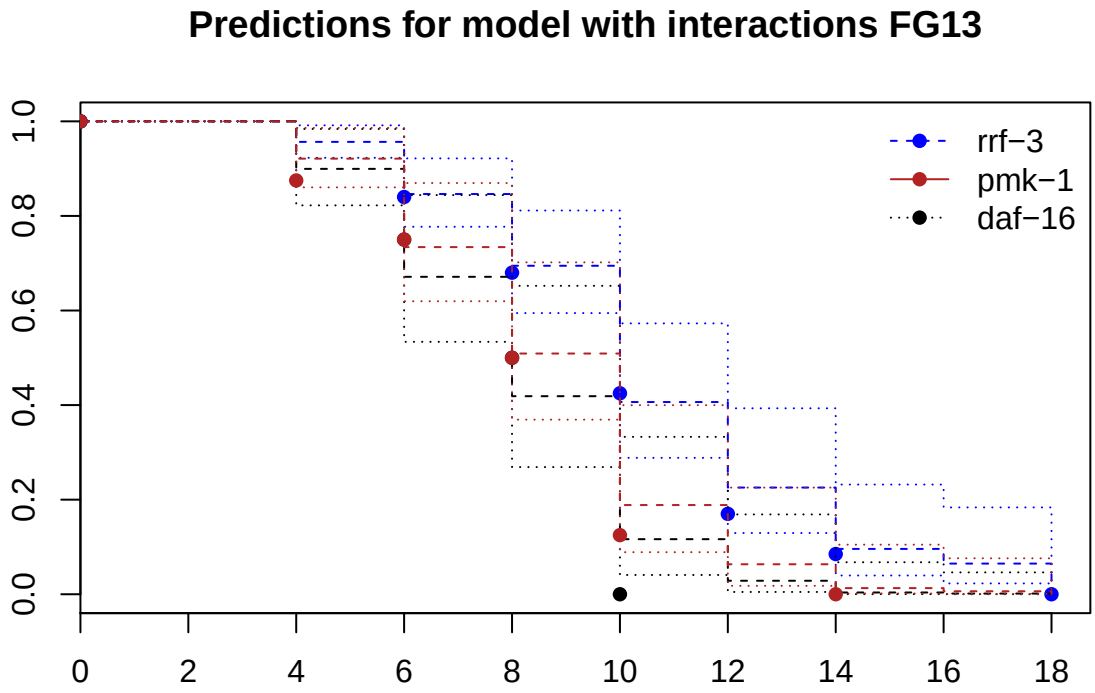
Fit is very bad as is clear both concordance and R^2 from the model. But there is far too little uncertainty in the confidence intervals. Which overestimates survival heavily for this group. Lets try the other model

```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'OP50')), lty = 2, xaxp = c(0,18,9),
     main = 'Predictions for model with interactions OP50')
points(df_OP50_r$Time,df_OP50_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'OP50')), lty = 2, col = 'black',)
points(df_OP50_d$Time,df_OP50_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'OP50')), lty = 2, col = 'firebrick
points(df_OP50_P$Time,df_OP50_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n', pch = 16)
```



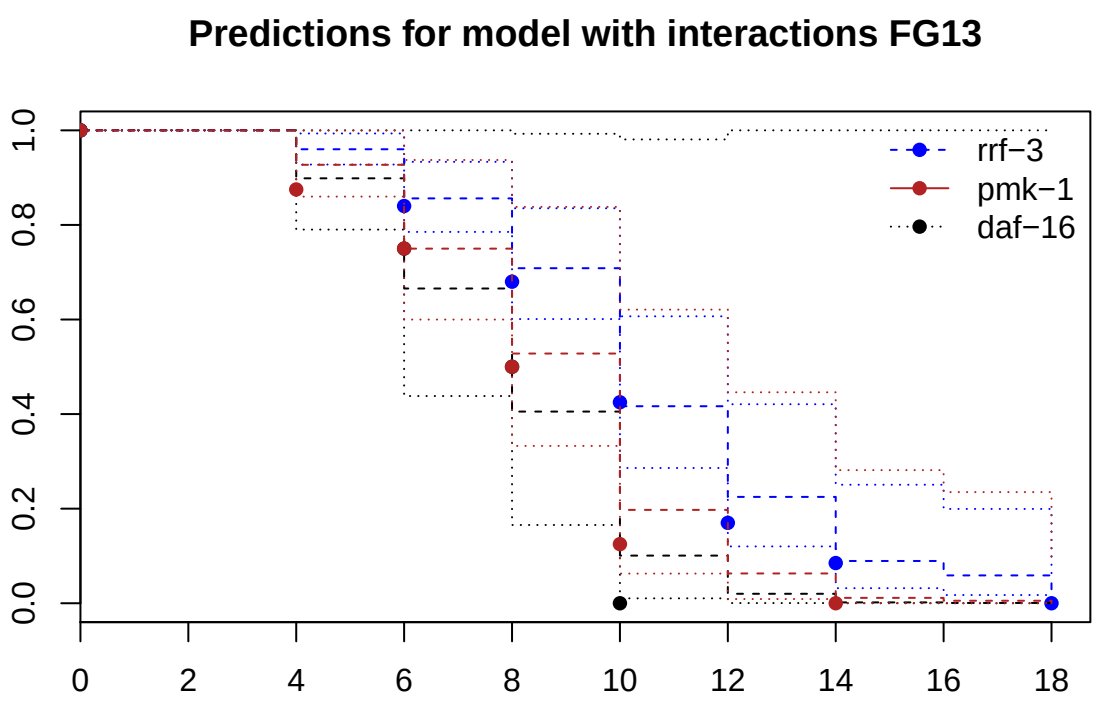
Still on the low end but a lot better. Seems that without interactions it overestimates survival on OP50.

```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'FG13')), lty = 2, xaxp = c(0,18,9)
     main = 'Predictions for model with interactions FG13')
points(df_fly_13_r$Time,df_fly_13_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'FG13')), lty = 2, col = 'black',)
points(df_fly_13_d$Time,df_fly_13_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'FG13')), lty = 2, col = 'firebric
points(df_fly_13_P$Time,df_fly_13_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n', pch = 16)
```



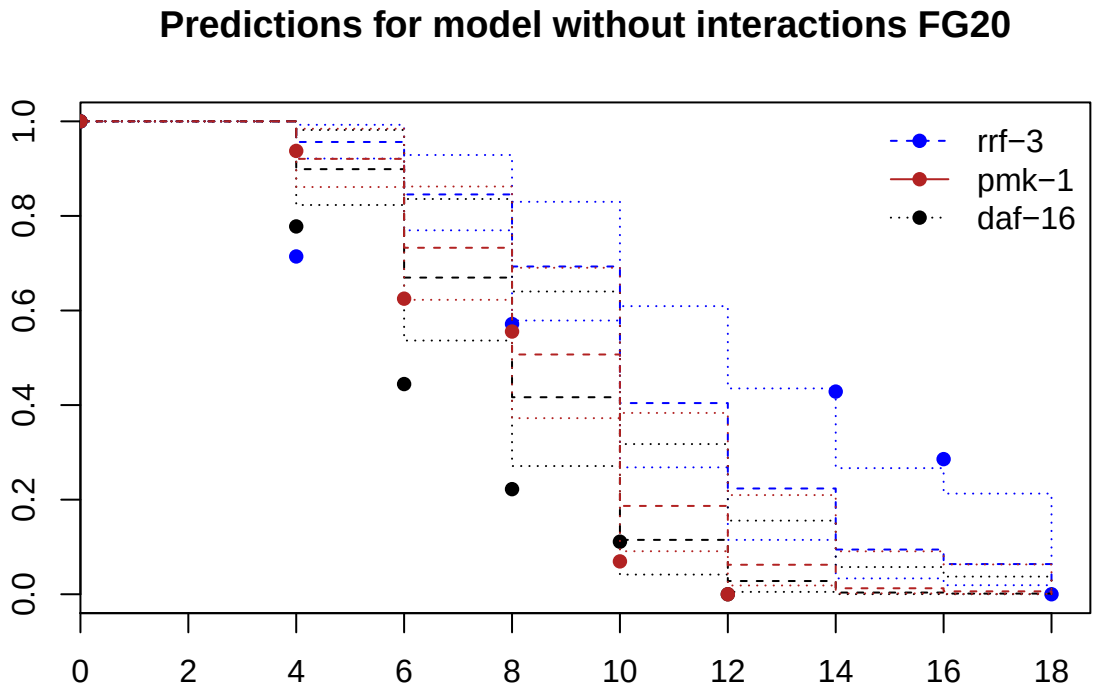
Fits notably better

```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'FG13')), lty = 2, xaxp = c(0,18,9),
     main = 'Predictions for model with interactions FG13')
points(df_fly_13_r$Time,df_fly_13_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'FG13')), lty = 2, col = 'black',)
points(df_fly_13_d$Time,df_fly_13_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'FG13')), lty = 2, col = 'firebrick
points(df_fly_13_P$Time,df_fly_13_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
        lty = c(2,1,3), col = c('blue','firebrick','black'),
        bty = 'n', pch = 16)
```



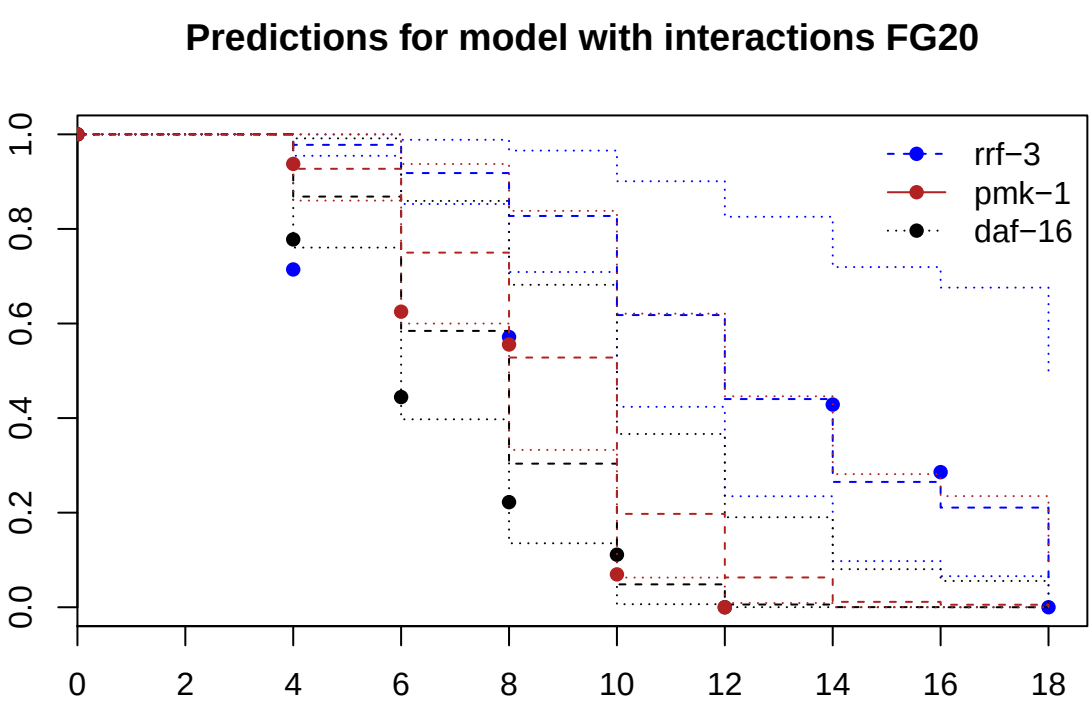
Change opacity on some points to fix overlap

```
plot(survfit(fit_both, newdata = data.frame(mutant = 'RRF', media = 'FG20')), lty = 2, xaxp = c(0,18,9)
     main = 'Predictions for model without interactions FG20')
points(df_fly_20_r$Time,df_fly_20_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'DAF', media = 'FG20')), lty = 2, col = 'black',)
points(df_fly_20_d$Time,df_fly_20_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_both, newdata = data.frame(mutant = 'PMK', media = 'FG20')), lty = 2, col = 'firebrick
points(df_fly_20_P$Time,df_fly_20_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
       lty = c(2,1,3), col = c('blue','firebrick','black'),
       bty = 'n', pch = 16)
```



Fitsvery bad with far too much uncertainty

```
plot(survfit(fit_int, newdata = data.frame(mutant = 'RRF', media = 'FG20')), lty = 2, xaxp = c(0,18,9),
     main = 'Predictions for model with interactions FG20')
points(df_fly_20_r$Time,df_fly_20_r$Surv, col = 'blue', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'DAF', media = 'FG20')), lty = 2, col = 'black',)
points(df_fly_20_d$Time,df_fly_20_d$Surv, col = 'black', pch = 16)
lines(survfit(fit_int, newdata = data.frame(mutant = 'PMK', media = 'FG13')), lty = 2, col = 'firebrick
points(df_fly_20_P$Time,df_fly_20_P$Surv, col = 'firebrick', pch = 16)
legend('topright', legend = c('rrf-3','pmk-1','daf-16'),
        lty = c(2,1,3), col = c('blue','firebrick','black'),
        bty = 'n', pch = 16)
```



The model has a problem with catching that all worms die around the same time and may therefore overestimate survival as it fits okay until the worms start dying. Some other model structure is maybe necessary or not all important covariates of survival are measured.