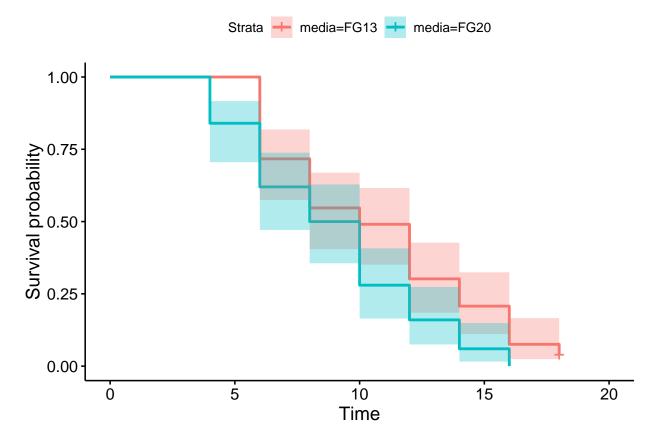
# Metabolite

## Jonas Gehrlein 29 nov 2018

```
data_met$Survobj <- with(data_met,Surv(data_met$Time, event = data_met$status))
d_OP50$Survobj <- with(d_OP50,Surv(d_OP50$Time, event = d_OP50$status))

km <- survfit(Survobj ~ media, data = data_met,conf.type = "log-log", error = "greenwood")
s_km <- summary(km)
ggsurvplot(km, conf.int = TRUE)</pre>
```

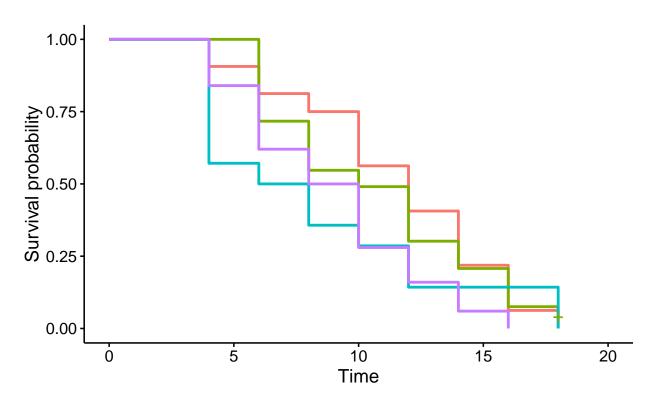


```
data_met_50 <- rbind(data_met, d_OP50)
fit <- coxph(Surv(Time, status) ~ media, data = data_met_50)
summary(fit)</pre>
```

```
## Call:
## coxph(formula = Surv(Time, status) ~ media, data = data met 50)
##
    n= 149, number of events= 139
##
##
                coef exp(coef) se(coef)
                                             z Pr(>|z|)
##
## mediaFG20 0.5076
                        1.6613
                                 0.2029 2.502 0.01235 *
                        0.5302
                                 0.2177 -2.915 0.00356 **
## mediaOP50 -0.6345
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
             exp(coef) exp(-coef) lower .95 upper .95
##
## mediaFG20
                1.6613
                            0.602
                                     1.1162
## mediaOP50
                0.5302
                            1.886
                                     0.3461
                                               0.8123
##
## Concordance= 0.639 (se = 0.025)
## Rsquare= 0.16
                   (max possible= 1 )
## Likelihood ratio test= 26.05 on 2 df,
                        = 25.11 on 2 df,
## Wald test
                                            p = 4e - 06
## Score (logrank) test = 26.78 on 2 df,
                                            p = 2e - 06
data_met_50$media <- factor(data_met_50$media, c('OP50','FG13','FG20'))
fit <- coxph(Surv(Time, status) ~ media, data = data_met_50)</pre>
summary(fit)
## coxph(formula = Surv(Time, status) ~ media, data = data_met_50)
##
##
    n= 149, number of events= 139
##
##
               coef exp(coef) se(coef)
                                           z Pr(>|z|)
## mediaFG13 0.6345
                      1.8861
                                0.2177 2.915 0.00356 **
## mediaFG20 1.1421
                       3.1333
                                0.2279 5.011 5.43e-07 ***
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
             exp(coef) exp(-coef) lower .95 upper .95
##
## mediaFG13
                 1.886
                           0.5302
                                      1.231
                                                 2.890
## mediaFG20
                 3.133
                           0.3191
                                      2.004
                                                 4.898
##
## Concordance= 0.639 (se = 0.025)
## Rsquare= 0.16
                   (max possible= 1 )
## Likelihood ratio test= 26.05 on 2 df,
                                            p = 2e - 06
## Wald test
                        = 25.11 on 2 df,
                                            p = 4e - 06
## Score (logrank) test = 26.78 on 2 df,
                                            p=2e-06
```

Alo lower survival for FG20 if only including survival and higher survival for OP50 which is higher than if we had compared with fly gut which isn't only metabolites. If we include metabolites in a  $\cos$  model without OP50

```
data_2_fly <- subset(data_2, media != 'OP50' )
data_2_fly$Survobj <- with(data_2_fly,Surv(data_2_fly$Time, event = data_2_fly$status))
km_fly <- survfit(Survobj ~ media + Metabolite, data = data_2_fly,conf.type = "log-log", error = "green ggsurvplot(km_fly)</pre>
```



No big difference on the plot between metabolite or without if we fit a model of both media and metabolite

```
fit_fly_meta <- coxph(Survobj ~ media + Metabolite , data = data_2_fly)
summary(fit_fly_meta)</pre>
```

```
## Call:
## coxph(formula = Survobj ~ media + Metabolite, data = data_2_fly)
##
    n= 149, number of events= 147
##
##
##
                 coef exp(coef) se(coef)
                                             z Pr(>|z|)
                                0.17022 2.915 0.00356 **
## mediaFG20 0.49618
                        1.64243
## mediaOP50
                   NA
                                0.00000
                                            NA
## Metabolite 0.08789
                        1.09186 0.18127 0.485 0.62778
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
##
              exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20
                  1.642
                            0.6089
                                      1.1765
                                                 2.293
## mediaOP50
                     NA
                                NA
                                                    NA
                                          NA
## Metabolite
                  1.092
                            0.9159
                                      0.7654
                                                 1.558
##
## Concordance= 0.59 (se = 0.027)
## Rsquare= 0.059
                   (max possible= 1 )
## Likelihood ratio test= 9.07 on 2 df,
                                           p=0.01
## Wald test
                        = 9.31 on 2 df,
                                           p=0.01
```

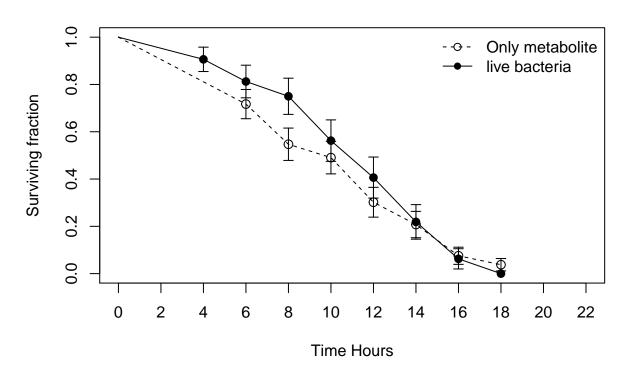
```
## Score (logrank) test = 9.5 on 2 df, p=0.009
AIC(fit_fly_meta)
## [1] 1193.565
fit_fly_med <- coxph(Survobj ~ media , data = data_2_fly)</pre>
summary(fit_fly_med)
## Call:
## coxph(formula = Survobj ~ media, data = data_2_fly)
##
##
    n= 149, number of events= 147
##
##
               coef exp(coef) se(coef)
                                            z Pr(>|z|)
## mediaFG20 0.5080
                       1.6619
                                0.1684 3.016 0.00256 **
## mediaOP50
                 NΑ
                           NA
                                0.0000
                                           NA
                                                    NΑ
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
             exp(coef) exp(-coef) lower .95 upper .95
                           0.6017
## mediaFG20
                 1.662
                                       1.195
## mediaOP50
                    NA
                                NA
                                          NA
                                                    NA
## Concordance= 0.586 (se = 0.025)
## Rsquare= 0.058
                    (max possible= 1 )
## Likelihood ratio test= 8.83 on 1 df,
                                            p=0.003
## Wald test
                        = 9.1 \text{ on } 1 \text{ df},
                                           p=0.003
## Score (logrank) test = 9.28 on 1 df,
                                           p=0.002
AIC(fit_fly_med)
```

#### ## [1] 1191.803

It certainly isn't a good predictor in the model. and is centered around 0. But very uncertain about the exact size on the size of the predictor. The only real difference is for FG13 where metabolite has worse survival

```
km_meta <- survfit(Survobj ~ media, data = data_meta,conf.type = "log-log", error = "greenwood")
s_km_meta <- summary(km_meta)</pre>
df_{13}_{meta} \leftarrow data.frame(c(0,s_km_meta$time[1:7]),c(1,s_km_meta$surv[1:7]),
                        c(0,s_km_meta$std.err[1:7]))
colnames(df_13_meta) <- c('Time', 'Surv', 'Std.error')</pre>
df_20_meta <- data.frame(c(0,s_km_meta$time[8:14]),c(1,s_km_meta$surv[8:14]),
                        c(0,s km meta\$std.err[8:14]))
colnames(df_20_meta) <- c('Time', 'Surv', 'Std.error')</pre>
plot(df_13_bac$Time[2:9],df_13_bac$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time Hours',
     ylab = 'Surviving fraction',
     main = expression('Comparision between FG13 without and with living bacteria'),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_13_bac$Time,df_13_bac$Surv)
arrows(df_13_bac$Time, df_13_bac$Surv-df_13_bac$Std.error, df_13_bac$Time,
       df_13_bac$Surv+df_13_bac$Std.error, length=0.05, angle=90, code=3, col = 'black')
## Warning in arrows(df_13_bac$Time, df_13_bac$Surv - df_13_bac$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
points(df_13_meta$Time[2:9],df_13_meta$Surv[2:9], pch = 1, cex = 1.2)
lines(df_13_meta$Time,df_13_meta$Surv, lty = 2)
arrows(df_13_meta$Time, df_13_meta$Surv-df_13_meta$Std.error, df_13_meta$Time, df_13_meta$Surv+df_13_me
## Warning in arrows(df_13_meta$Time, df_13_meta$Surv -
## df 13 meta$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
legend('topright', pch = c(1,16), lty = c(2,1), bty = 'n',
       legend = c('Only metabolite','live bacteria'))
```

### Comparision between FG13 without and with living bacteria

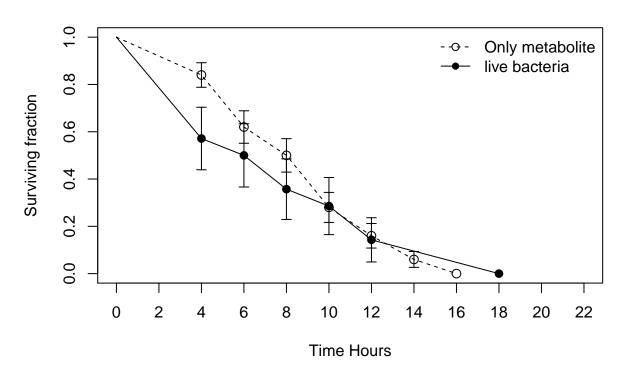


```
plot(df_20_bac$Time[2:9],df_20_bac$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time Hours',
    ylab = 'Surviving fraction',
    main = expression('Comparision between FG20 without and with living bacteria'),
    xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_20_bac$Time,df_20_bac$Surv)
arrows(df_20_bac$Time[2:9], df_20_bac$Surv[2:9]-df_20_bac$Std.error[2:9], df_13_bac$Time[2:9],
    df_20_bac$Surv[2:9]+df_20_bac$Std.error[2:9], length=0.05, angle=90, code=3, col = 'black')
points(df_20_meta$Time[2:9],df_20_meta$Surv[2:9], pch = 1, cex = 1.2)
lines(df_20_meta$Time,df_20_meta$Surv, lty = 2)
arrows(df_20_meta$Time,df_20_meta$Surv, lty = 2)
arrows(df_20_meta$Time, df_20_meta$Surv-df_20_meta$Std.error, df_20_meta$Time, df_20_meta$Surv+df_20_me

## Warning in arrows(df_20_meta$Time, df_20_meta$Surv -
## df_20_meta$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped

legend('topright', pch = c(1,16), lty = c(2,1), bty = 'n',
    legend = c('Only metabolite','live bacteria'))
```

#### Comparision between FG20 without and with living bacteria



```
d_0P50$Survobj <- with(d_0P50,Surv(d_0P50$Time, event = d_0P50$status))</pre>
km_OP50 <- survfit(Survobj ~ media, data = d_OP50,conf.type = "log-log", error = "greenwood")
s_km_OP50 <- summary(km_OP50)</pre>
df_OP50 <- data.frame(c(0,s_km_OP50$time[1:8]),c(1,s_km_OP50$surv[1:8]),
                        c(0,s_km_OP50$std.err[1:8]))
colnames(df_OP50) <- c('Time','Surv','Std.error')</pre>
plot(df_13_bac$Time[2:9],df_13_bac$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time (hours)',
     ylab = 'Surviving fraction', col = 'blue',
     main = expression('Heat knockdown survival of' ~ italic(C.)~italic(elegans)~ 'replicate 2'),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_13_bac$Time,df_13_bac$Surv, col = 'blue')
arrows(df 13 bac$Time, df 13 bac$Surv-df 13 bac$Std.error, df 13 bac$Time,
       df_13_bac$Surv+df_13_bac$Std.error, length=0.05, angle=90, code=3,
       col = 'blue')
## Warning in arrows(df_13_bac$Time, df_13_bac$Surv - df_13_bac$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
points(df_13_meta$Time[2:9],df_13_meta$Surv[2:9], pch = 1, cex = 1.2,
       col = 'blue' )
lines(df_13_meta$Time,df_13_meta$Surv, lty = 2, col = 'blue')
arrows(df 13 meta$Time, df 13 meta$Surv-df 13 meta$Std.error, df 13 meta$Time, df 13 meta$Surv+df 13 me
       col = 'blue')
```

## Warning in arrows(df 13 meta\$Time, df 13 meta\$Surv -

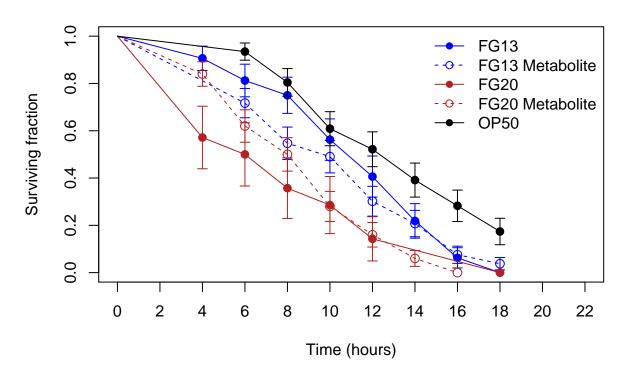
```
## skipped
points(df_20_bac$Time[2:9],df_20_bac$Surv[2:9], pch = 16, cex = 1.2,
       col = 'firebrick')
lines(df_20_bac$Time,df_20_bac$Surv,col = 'firebrick')
arrows(df_20_bac$Time[2:9], df_20_bac$Surv[2:9]-df_20_bac$Std.error[2:9], df_20_bac$Time[2:9],
       df_20_bac$Surv[2:9]+df_20_bac$Std.error[2:9], length=0.05, angle=90, code=3, col = 'firebrick')
points(df_20_meta$Time[2:9],df_20_meta$Surv[2:9], pch = 1, cex = 1.2,
       col = 'firebrick')
lines(df_20_meta$Time,df_20_meta$Surv, lty = 2,col = 'firebrick')
arrows(df_20_meta$Time, df_20_meta$Surv-df_20_meta$Std.error, df_20_meta$Time, df_20_meta$Surv+df_20_me
       col = 'firebrick')
## Warning in arrows(df_20_meta$Time, df_20_meta$Surv -
## df_20_meta$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
points(df OP50$Time[2:9], df OP50$Surv[2:9], pch = 16, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 1)
arrows(df_0P50$Time, df_0P50$Surv-df_0P50$Std.error, df_0P50$Time, df_0P50$Surv+df_0P50$Std.error, leng
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
legend('topright', pch = c(16,1,16,1,16), lty = c(1,2,1,2,1), bty = 'n',
       col = c('blue','blue','firebrick','firebrick', 'black'),
```

## df\_13\_meta\$Std.error, : zero-length arrow is of indeterminate angle and so

legend = c('FG13','FG13 Metabolite','FG20','FG20 Metabolite',

'OP50'))

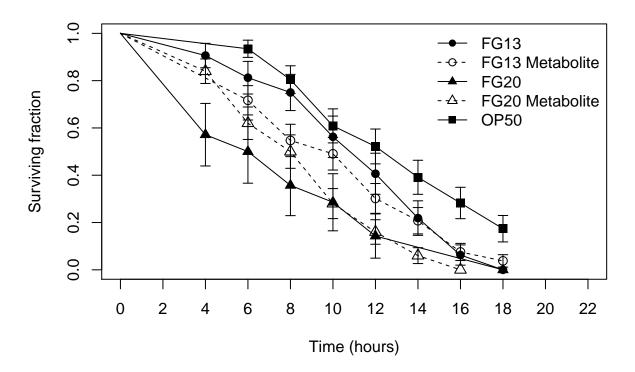
### Heat knockdown survival of C. elegans replicate 2



```
plot(df_13_bac$Time[2:9],df_13_bac$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time (hours)',
     ylab = 'Surviving fraction',
     main = expression('Heat knockdown survival of'
                       ~ italic(C.)~italic(elegans)~ 'replicate 2'),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_13_bac$Time,df_13_bac$Surv)
arrows(df_13_bac$Time, df_13_bac$Surv-df_13_bac$Std.error, df_13_bac$Time,
       df_13_bac$Surv+df_13_bac$Std.error, length=0.05, angle=90, code=3)
## Warning in arrows(df_13_bac$Time, df_13_bac$Surv - df_13_bac$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
points(df_13_meta$Time[2:9],df_13_meta$Surv[2:9], pch = 1, cex = 1.2)
lines(df_13_meta$Time,df_13_meta$Surv, lty = 2)
arrows(df_13_meta$Time, df_13_meta$Surv-df_13_meta$Std.error, df_13_meta$Time, df_13_meta$Surv+df_13_me
## Warning in arrows(df_13_meta$Time, df_13_meta$Surv -
## df_13_meta$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
points(df_20_bac$Time[2:9],df_20_bac$Surv[2:9], pch = 17, cex = 1.2)
lines(df_20_bac$Time,df_20_bac$Surv)
arrows(df 20 bac$Time[2:9], df 20 bac$Surv[2:9]-df 20 bac$Std.error[2:9], df 20 bac$Time[2:9],
```

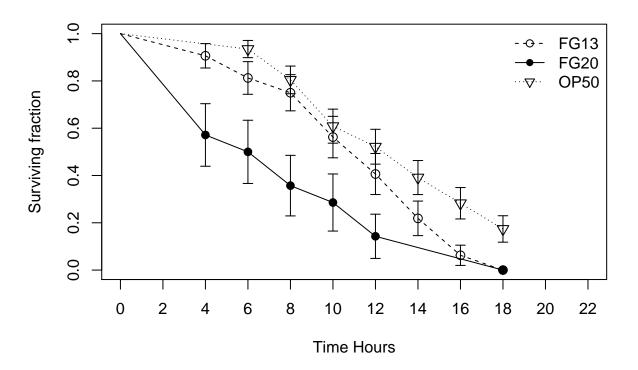
df\_20\_bac\$Surv[2:9]+df\_20\_bac\$Std.error[2:9], length=0.05, angle=90, code=3)

## Heat knockdown survival of C. elegans replicate 2



```
plot(df_20_bac$Time[2:9],df_20_bac$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time Hours',
    ylab = 'Surviving fraction',
    main = expression('Comparision between FG20 and FG13'),
    xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
```

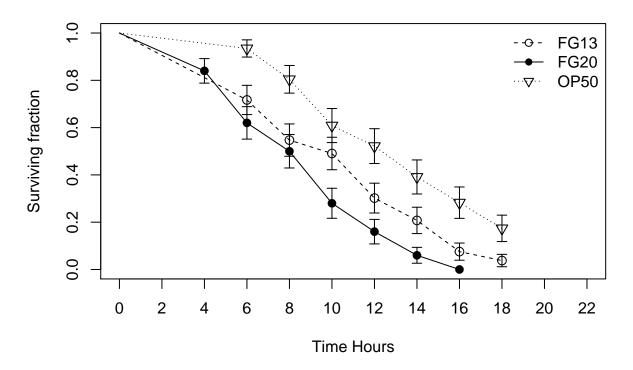
#### Comparision between FG20 and FG13



```
plot(df_20_meta$Time[2:9],df_20_meta$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Time Hours',
    ylab = 'Surviving fraction',
    main = expression('Comparision between FG20 and FG13 for Metabolite'),
```

```
xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_20_meta$Time,df_20_meta$Surv)
arrows(df_20_meta$Time[2:9], df_20_meta$Surv[2:9]-df_20_meta$Std.error[2:9], df_20_meta$Time[2:9],
       df_20_meta$Surv[2:9]+df_20_meta$Std.error[2:9], length=0.05, angle=90, code=3, col = 'black')
points(df_13_meta$Time[2:9],df_13_meta$Surv[2:9], pch = 1, cex = 1.2)
lines(df_13_meta$Time,df_13_meta$Surv, lty = 2)
arrows(df_13_meta$Time, df_13_meta$Surv-df_13_meta$Std.error, df_13_meta$Time, df_13_meta$Surv+df_13_me
## Warning in arrows(df_13_meta$Time, df_13_meta$Surv -
## df_13_meta$Std.error, : zero-length arrow is of indeterminate angle and so
## skipped
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 6, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 3)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error, df_OP50$Time, df_OP50$Surv+df_OP50$Std.error, leng
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
legend('topright', pch = c(1,16,6), lty = c(2,1,3), bty = 'n',
       legend = c('FG13','FG20','OP50'))
```

## Comparision between FG20 and FG13 for Metabolite



```
d_13_m <- subset(data_meta,media == 'FG13')</pre>
d_20_m <- subset(data_meta, media == 'FG20')</pre>
d_1350_m <- rbind(d_13_m, d_0P50)</pre>
d_2050_m <- rbind(d_20_m,d_0P50)</pre>
d_13_b <- subset(data_bac, media =='FG13')</pre>
d_20_b <- subset(data_bac, media =='FG20')</pre>
d_13_bm <- rbind(d_13_m,d_13_b)</pre>
d_20_bm <- rbind(d_20_b,d_20_m)</pre>
survdiff(Survobj ~ media, data = data_meta)
## Call:
## survdiff(formula = Survobj ~ media, data = data_meta)
##
##
               N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 53
                        51
                                61.3
                                          1.74
                                39.7
## media=FG20 50
                        50
                                          2.69
                                                     6.56
##
## Chisq= 6.6 on 1 degrees of freedom, p= 0.01
survdiff(Surv(Time, status) ~ media, data = d_1350_m)
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_1350_m)
##
##
               N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 53
                        51
                                39.3
                                          3.49
                                                      8.4
## media=0P50 46
                        38
                                49.7
                                          2.76
                                                      8.4
##
   Chisq= 8.4 on 1 degrees of freedom, p= 0.004
survdiff(Surv(Time, status) ~ media, data = d_2050_m)
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_2050_m)
               N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG20 50
                        50
                                31.2
                                         11.38
                                                     24.7
## media=0P50 46
                        38
                                56.8
                                          6.24
                                                     24.7
##
## Chisq= 24.7 on 1 degrees of freedom, p= 7e-07
fit_13_mb <- coxph(Surv(Time, status) ~Metabolite, data = d_13_bm)</pre>
summary(fit_13_mb)
## Call:
## coxph(formula = Surv(Time, status) ~ Metabolite, data = d_13_bm)
##
##
     n= 85, number of events= 83
##
                  coef exp(coef) se(coef)
                                               z Pr(>|z|)
## Metabolite 0.05413    1.05562    0.22642    0.239
```

```
##
            exp(coef) exp(-coef) lower .95 upper .95
              1.056
                        0.9473
                                0.6773
## Metabolite
##
## Concordance= 0.529 (se = 0.035 )
## Rsquare= 0.001 (max possible= 0.999)
## Likelihood ratio test= 0.06 on 1 df, p=0.8
                     = 0.06 on 1 df,
## Wald test
                                      p=0.8
## Score (logrank) test = 0.06 on 1 df, p=0.8
fit_20_mb <- coxph(Surv(Time, status) ~Metabolite, data = d_20_bm)</pre>
summary(fit_20_mb)
## Call:
## coxph(formula = Surv(Time, status) ~ Metabolite, data = d_20_bm)
## n= 64, number of events= 64
##
##
              coef exp(coef) se(coef) z Pr(>|z|)
exp(coef) exp(-coef) lower .95 upper .95
              1.11 0.9013
                                0.5835
## Metabolite
## Concordance= 0.465 (se = 0.04)
## Rsquare= 0.002 (max possible= 0.998)
## Likelihood ratio test= 0.1 on 1 df, p=0.7
## Wald test
              = 0.1 \text{ on } 1 \text{ df},
                                     p = 0.8
## Score (logrank) test = 0.1 on 1 df, p=0.8
```