KM plots based on TBXT mRNA levels

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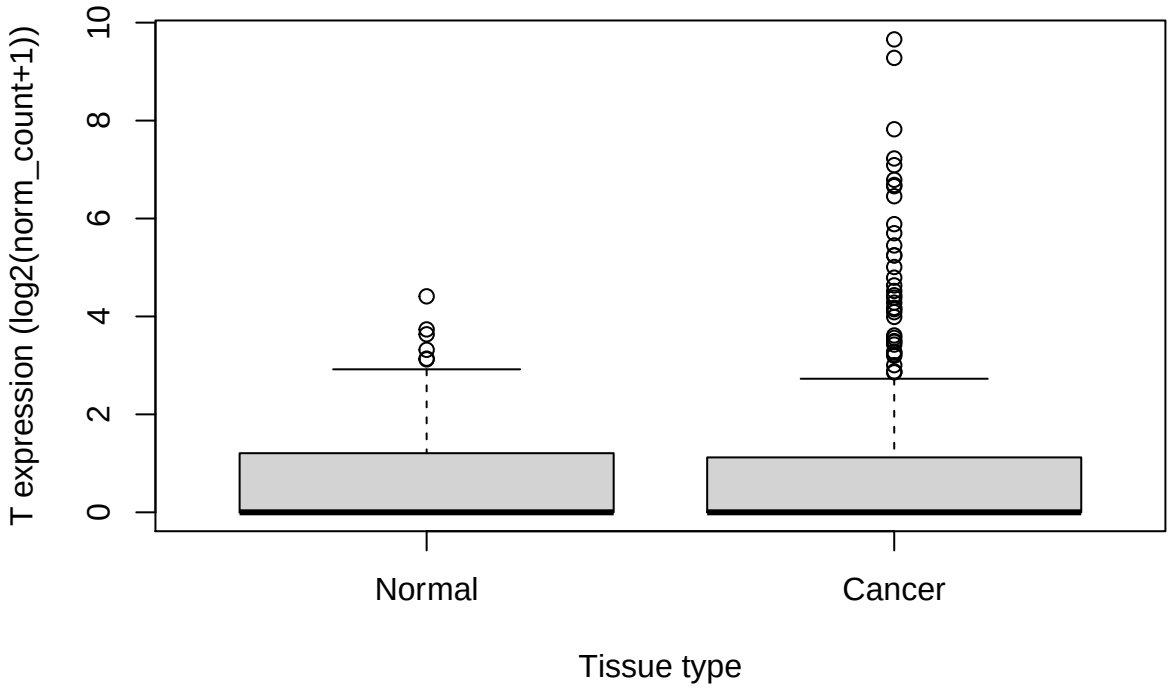
Boxplot shows distributuon of data

This is a boxplot summarising TBXT mRNA levels in normal colon tissue (GTex) vs primary colorectal adenoma (TCGA).

```
normal <- filter(samples, X_sample_type == "Normal Tissue")
cancer <- filter(samples, X_sample_type == "Primary Tumor")
boxplot(normal$T, cancer$T,
        at = c(1,2),
        names = c("Normal", "Cancer"),
        xlab = "Tissue type",
        ylab = "T expression (log2(norm_count+1))")
```



As you can see from the boxplot although the majority of samples in both datasets are similarly distributed with no/ low TBXT mRNA, the cancer dataset has more outliers that exhibit higher TBXT expression.

Survival analyses (Kaplan Meier plots)

These analyses define a high Brachyury group and a low Brachyury group, look at the relative survival of those groups then test for a significant different in survival using a logrank test. You get different results depending on how you define the groups - where you put the cut-off between low and high. Here I go through

what the results are for lots of different definitions.

Above the max normal value

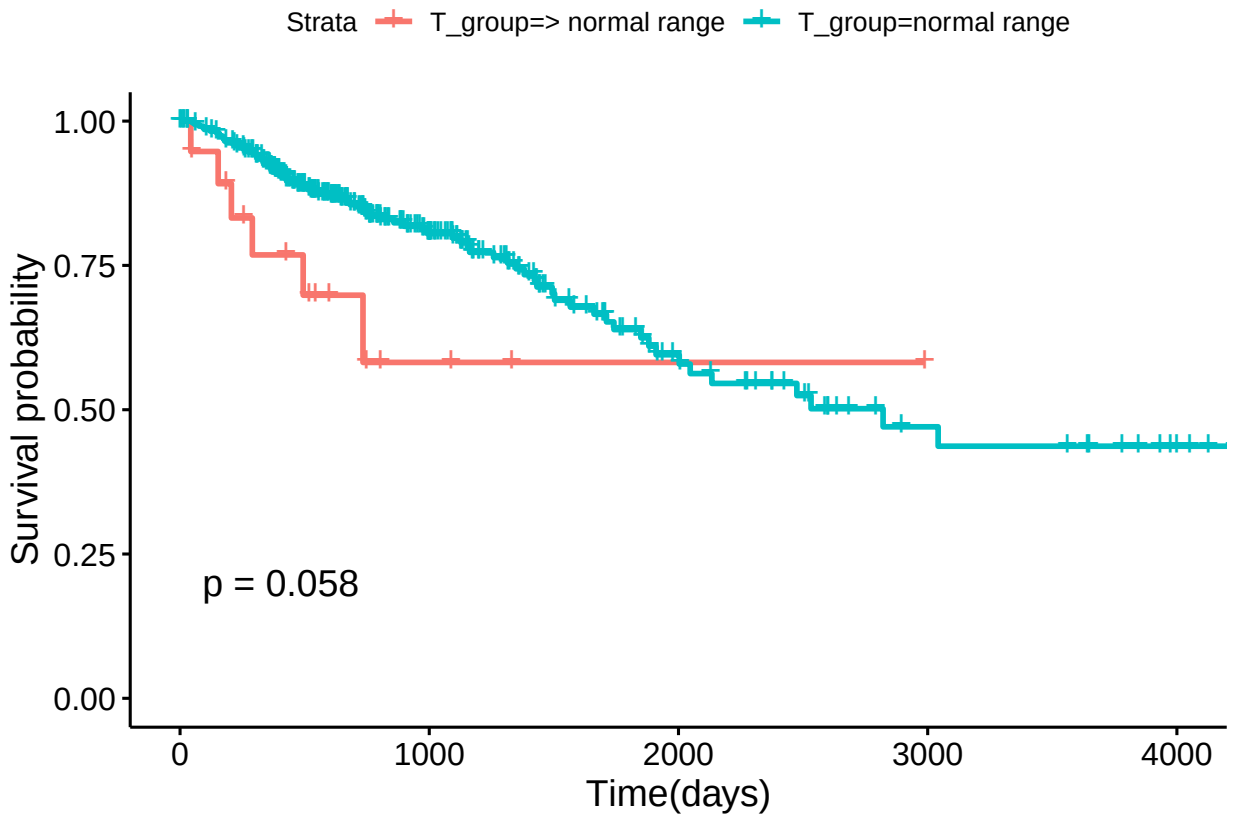
The max value for TBXT expression in the normal tissues (top dot in the boxplot) is:

```
max(normal$T)
```

```
## [1] 4.411
```

If we define 'High Brachyury' to be > 4.411 the analysis looks like this.

```
cancer <- cancer %>%
  mutate(T_group = ifelse(T > 4.4110, "> normal range","normal range"))
cancer$T_group <- factor(cancer$T_group)
surv_object <- Surv(time = cancer$OS.time, event = cancer$OS)
fit1 <- survfit(surv_object ~ T_group, data = cancer)
ggsurvplot(fit1, data = cancer, pval = TRUE, xlab = "Time(days)")
```



There appears to be a difference between the groups but it narrowly misses significance in the log-rank test. This table shows the number of people in each group and the number of 'events' (by which it means death).

```
fit1
```

```
## Call: survfit(formula = surv_object ~ T_group, data = cancer)
##
##    3 observations deleted due to missingness
##                           n events median 0.95LCL 0.95UCL
## T_group=> normal range   19      6     NA     734      NA
## T_group=normal range    358     79   2821    2003      NA
```

Top quartile (25%)

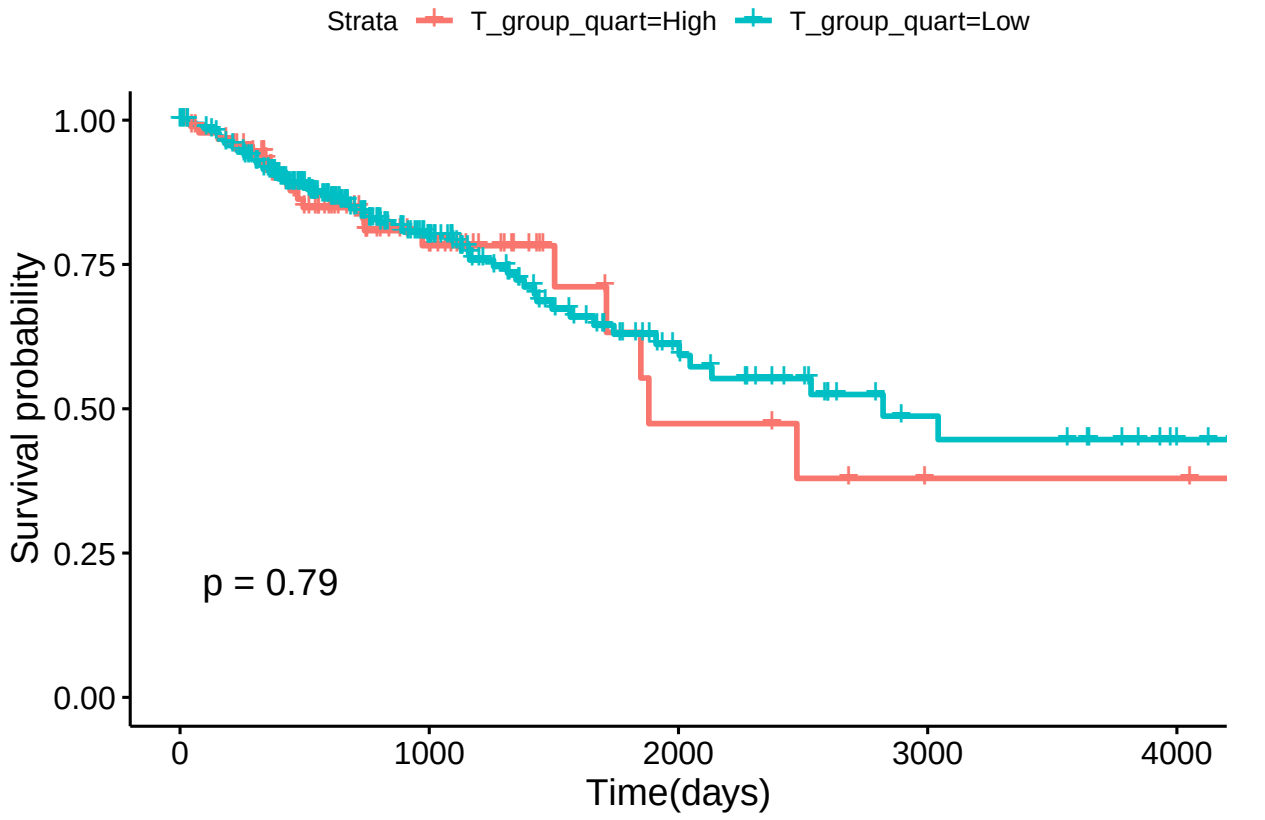
When we define 'High Brachyury' as the top quartile the analysis is as follows. Find top quartile:

```
summary(cancer$T)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.0000  0.0000  0.0000  0.8551  1.1160  9.6580
```

Make the Kaplan-Meier plot:

```
cancer <- cancer %>%
  mutate(T_group_quart = ifelse(T >= 1.1160, "High","Low"))
cancer$T_group_quart <- factor(cancer$T_group_quart)
surv_object <- Surv(time = cancer$OS.time, event = cancer$OS)
fit1 <- survfit(surv_object ~ T_group_quart, data = cancer)
ggsurvplot(fit1, data = cancer, pval = TRUE, xlab = "Time(days)")
```




```
fit1
```

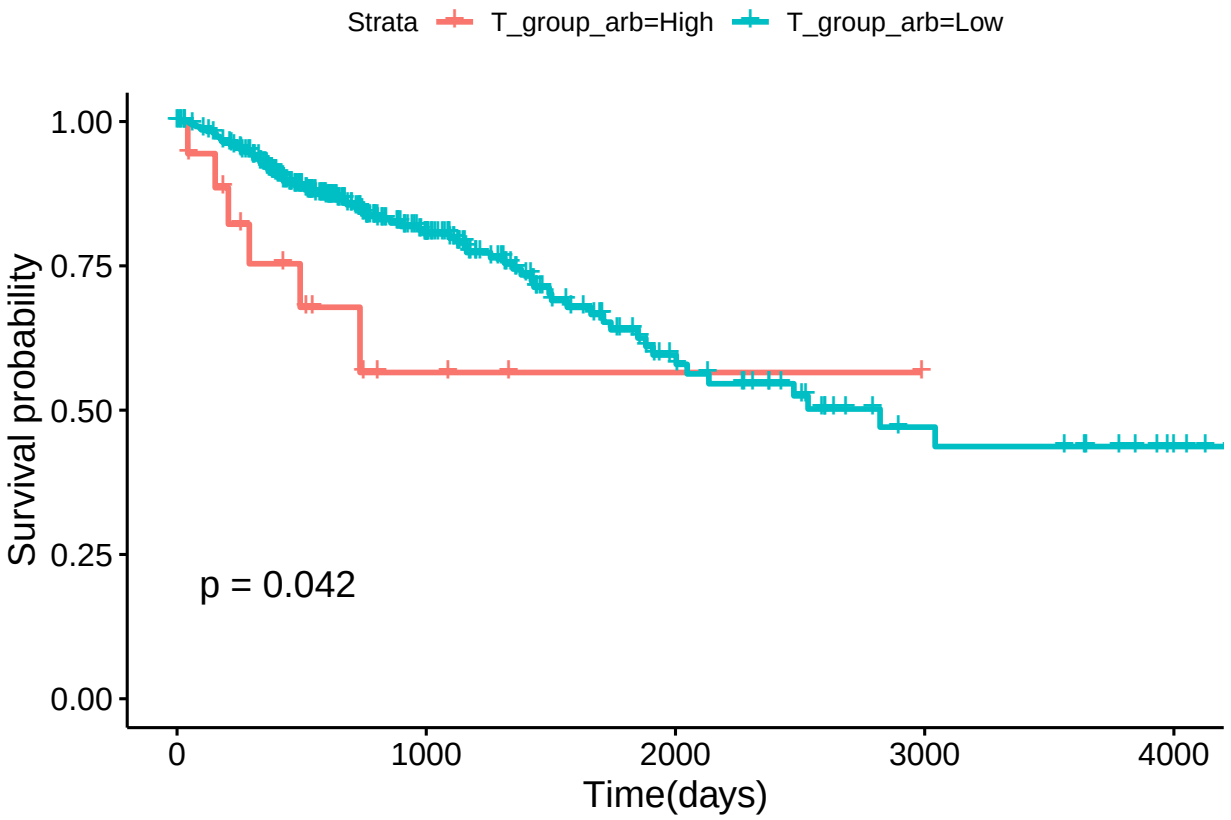
```
## Call: survfit(formula = surv_object ~ T_group_quart, data = cancer)
##
##    3 observations deleted due to missingness
##                       n events median 0.95LCL 0.95UCL
## T_group_quart=High  93     20   1881    1711      NA
## T_group_quart=Low  284     65   2821    2047      NA
```

There is no difference between the groups. I don't think this is particularly suprising because lots of these samples have TBXT values well within the range of the normal tissues.

Arbitary cut-offs

So, starting from the cut-off of 4.4110 what happens if we bump the cut-off upwards slightly? First to 4.5.

```
cancer <- cancer %>%
  mutate(T_group_arb = ifelse(T > 4.5, "High","Low"))
cancer$T_group_arb <- factor(cancer$T_group_arb)
surv_object <- Surv(time = cancer$OS.time, event = cancer$OS)
fit1 <- survfit(surv_object ~ T_group_arb, data = cancer)
ggsurvplot(fit1, data = cancer, pval = TRUE, xlab = "Time(days)")
```



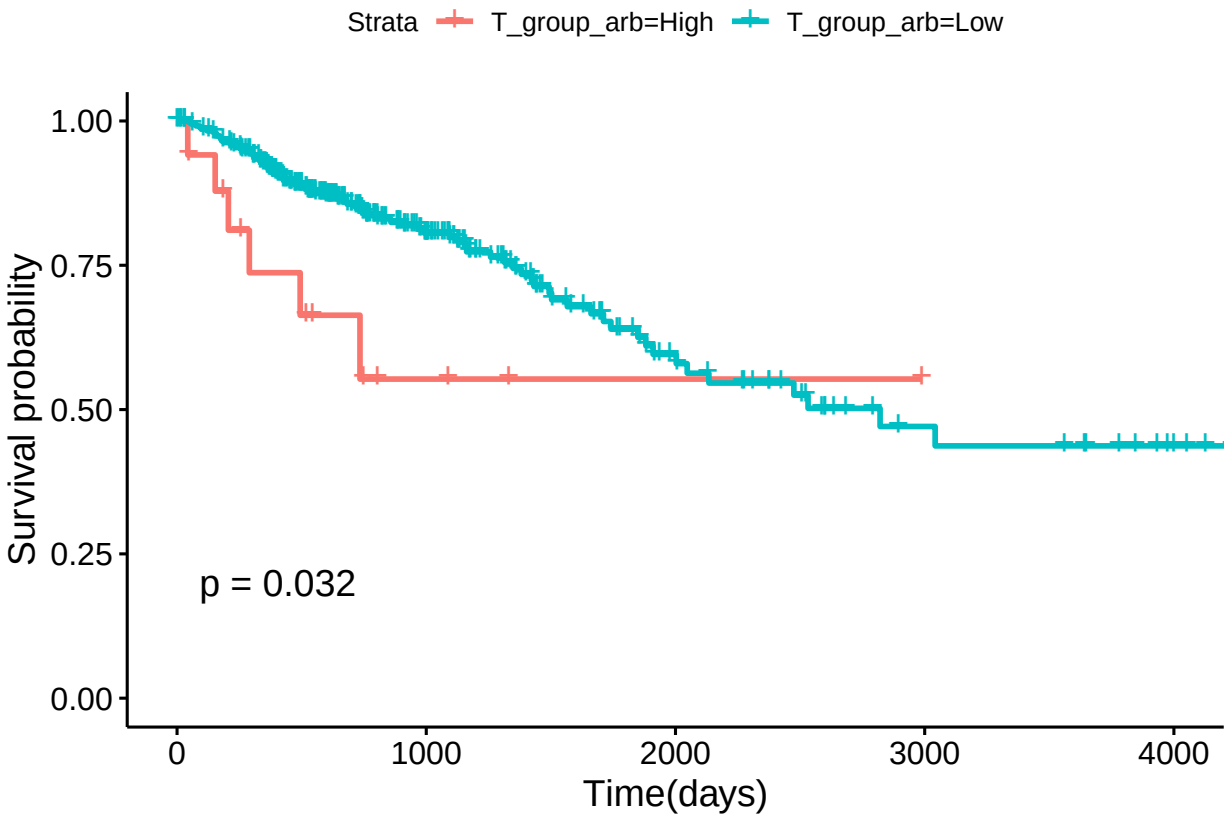

```
fit1
```

```
## Call: survfit(formula = surv_object ~ T_group_arb, data = cancer)
##
##    3 observations deleted due to missingness
##                     n events median 0.95LCL 0.95UCL
## T_group_arb=High   18      6     NA     494      NA
## T_group_arb=Low   359     79   2821    2003      NA
```

This loses only 1 patient from the high group and the results are now significant.

Using 4.6 as a cut-off we 'lose' one more patient from the 'High' group and it looks like this:

```
cancer <- cancer %>%
  mutate(T_group_arb = ifelse(T > 4.6, "High","Low"))
cancer$T_group_arb <- factor(cancer$T_group_arb)
surv_object <- Surv(time = cancer$OS.time, event = cancer$OS)
fit1 <- survfit(surv_object ~ T_group_arb, data = cancer)
ggsurvplot(fit1, data = cancer, pval = TRUE, xlab = "Time(days)")
```




```
fit1
```

```
## Call: survfit(formula = surv_object ~ T_group_arb, data = cancer)
##
##    3 observations deleted due to missingness
##                     n events median 0.95LCL 0.95UCL
## T_group_arb=High   17      6     NA     494      NA
## T_group_arb=Low   360     79   2821    2003      NA
```

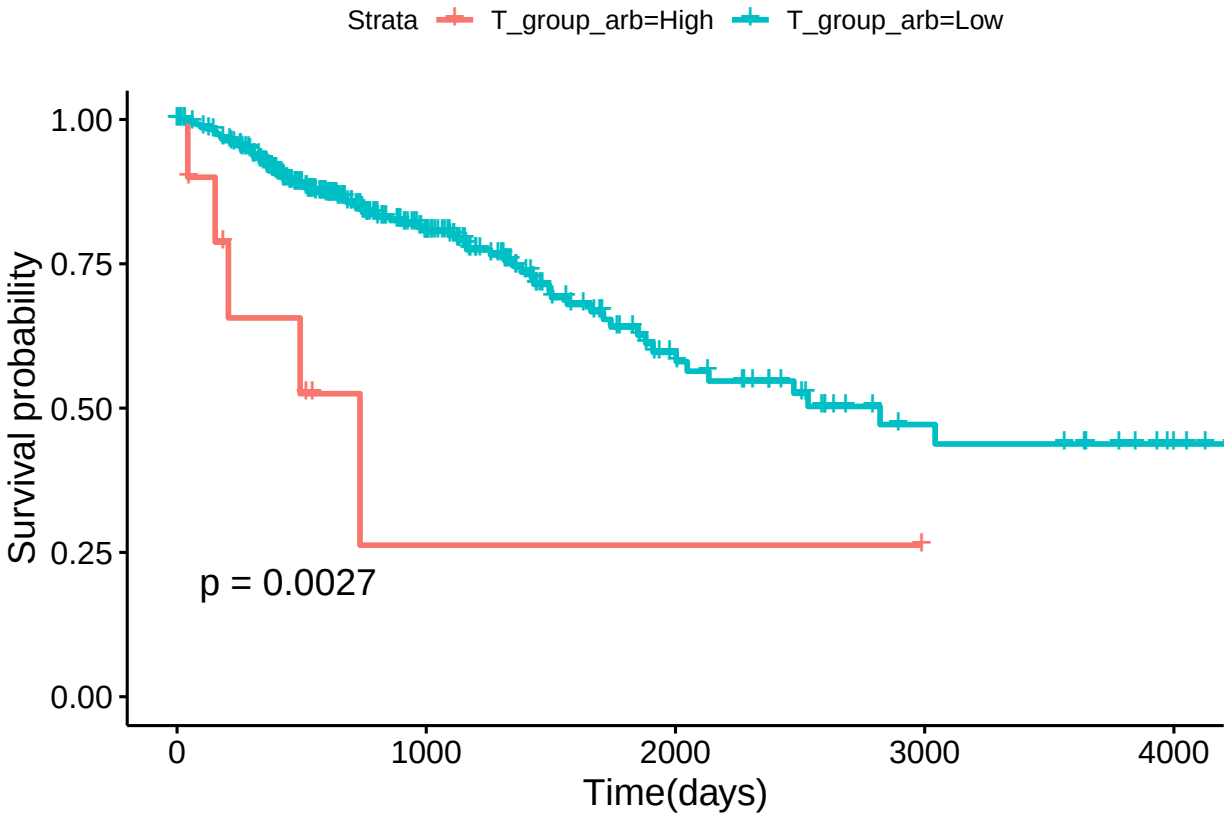
There is a more significant difference between the groups.

If I do this for lots of different arbitary cutoffs (between high and low Brachyury) - the P value changes as follows:

Cut-off	P value	n	Events
4.411	0.58	19	6
4.5	0.042	18	6
4.6	0.032	17	6
4.7	0.12	16	5
4.8	0.078	15	5
5.1	0.056	14	5
5.3	0.02	12	5
5.5	0.0075	11	5
5.7	0.0027	10	5
5.9	0.026	9	4
6.5	0.16	8	3
6.7	0.39	6	2

The cutoff which gives the lowest P value is 5.7 and the plot looks like this.

```
cancer <- cancer %>%
  mutate(T_group_arb = ifelse(T > 5.7, "High","Low"))
cancer$T_group_arb <- factor(cancer$T_group_arb)
surv_object <- Surv(time = cancer$OS.time, event = cancer$OS)
fit1 <- survfit(surv_object ~ T_group_arb, data = cancer)
ggsurvplot(fit1, data = cancer, pval = TRUE, xlab = "Time(days)")
```




```
fit1
```

```
## Call: survfit(formula = surv_object ~ T_group_arb, data = cancer)
##
##    3 observations deleted due to missingness
##                     n events median 0.95LCL 0.95UCL
## T_group_arb=High   10      5    734     206      NA
## T_group_arb=Low   367     80   2821    2003      NA
```

Conclusion

There does seem to be a difference in prognosis between patients with >normal and normal levels of TBXT mRNA. The observation of a difference seems to be robust at many different cut-offs, but the level of statistical significance varies depending on the cut-off.