

# R code used to generate figures in the manuscript: Topological Tumor Graphs: a graph-based spatial model to infer stromal recruitment for immunosuppression in melanoma histology

load data

```
load("TTG_data.Rdata")
```

## Creating figures

Figure 3a

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("RTCGA.clinical")
library(RTCGA.clinical)
install.packages("tidyverse")
library(tidyverse)
install.packages("survival")
library(survival)
install.packages("survminer")
library(survminer)
install.packages("survMisc")
library(survMisc)
install.packages("ggplot2")
library(ggplot2)
install.packages("ggpubr")
library(ggpubr)
install.packages("magrittr")
library(magrittr)
```

```
### Create quartiles of stromal barrier
```

```
TTG_data$strmbar_quartile <- with(TTG_data, cut(strml_barrier,
breaks=quantile(strml_barrier, probs=seq(0,1,0.25)),
include.lowest=TRUE,
labels = 1:4)
)
```

```
TTG_data$strmbar_quartile2only [strmbar_quartile == "1"] <- "Low barrier"
TTG_data$strmbar_quartile2only [strmbar_quartile==" 4"] <- "High barrier"
```

```
### univariate Cox-Proportional Hazards test for 1st and 4th quartiles of stromal barrier
```

```
univarcoc_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile, data=TTG_data)
summary(univarcoc_barrierquartile)
```

```
### survival plot for stromal barrier using the quartile cutoff described above
```

```
sfit2 <- survfit(Surv(times, patient.vital_status)~ strmbar_quartile2only, data=TTG_data)
```

```

colorpal3 = c("firebrick1", "royalblue3")

ggsurv2 <- ggsurvplot(sfit2, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650), break.time.by=1000, leg
  palette = colorpal3,
  ylab= "Overall Survival",
  title = "Stromal Barrier",
  font.title = c(20, "plain"),
  legend.labs = c ("high barrier", "low barrier"),
  font.x = c(20, "plain"),
  font.y = c(20, "plain"),
  font.tickslab = c(16, "plain"),
  tables.col = "strata",
  tables.y.text = FALSE,
  legend.title = "",
)

ggsurv2$plot <- ggsurv2$plot +
  theme(legend.text = element_text(size = 14, color = "black")) +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))

### multivariate
### adjust for stromal %
adj_strmpercent_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile + strm_per
summary(adj_strmpercent_barrierquartile)
### adjust for lymphocyte %
adj_lympercent_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile + lym_perce
summary(adj_lympercent_barrierquartile)

```

Figure 3b

```

### defining low and high stromal clustering using pre-defined threshold of 0.590253
TTG_data$strml_clustering_hcateg [TTG_data$strml_clustering > 0.590253] <- "2"
TTG_data$strml_clustering_hcateg [TTG_data$strml_clustering <= 0.590253] <- "1"

### univariate Cox-Proportional Hazards test for categorical stromal clustering groups
univarcox_clustering_hcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg, data=
summary(univarcox_clustering_hcateg)

### survival plot for stromal clustering using henrik's threshold
sfit1 <- survfit(Surv(time, status)~ TTG_data$strml_clustering_hcateg, data=TTG_data)
colorpal3 = c("firebrick1", "royalblue3")

ggsurv1 <- ggsurvplot(sfit1, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650), break.time.by=1000, leg
  palette = colorpal3,
  title = "Stromal Clustering",
  font.title = c(20, "plain"),
  ylab= "Overall Survival",

```

```

        legend.labs = c ("high clustering", "low clustering"),
        font.x = c(20, "plain"),
        font.y = c(20, "plain"),
        font.tickslab = c(16, "plain"),
        tables.col = "strata",
        tables.y.text = FALSE,
        legend.title = "",
    )

ggsurv1$plot <- ggsurv1$plot +
  theme(legend.text = element_text(size = 14, color = "black")) +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))

### multivariate
### adjust for strml %
adjstrmpercent_clustering_hcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg +
summary(adjstrmpercent_clustering_hcateg)
### adjust for lymphocyte %
adjlympercent_clustering_hcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg +
summary(adjlympercent_clustering_hcateg)

```

Figure 3c

```

### scatter plot of stromal barrier versis clustering
### create groups so that the 'missing' values (2nd and 3rd quartiles) are in grey
TTG_data$clustbar_combo [strml_clustering_hcateg=="1" & strmbar_quartile=="1"] <- "Low cluster/ Low ba
TTG_data$clustbar_combo [strml_clustering_hcateg=="1" & strmbar_quartile=="4"] <- "Low cluster/ high ba
TTG_data$clustbar_combo [strml_clustering_hcateg=="2" & strmbar_quartile=="1"] <- "high cluster/ low ba
TTG_data$clustbar_combo [strml_clustering_hcateg=="2" & strmbar_quartile=="4"] <- "high cluster/ high ba
TTG_data$clustbar_combo [strml_clustering_hcateg=="1" & strmbar_quartile=="2"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg=="1" & strmbar_quartile=="3"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg=="2" & strmbar_quartile=="2"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg=="2" & strmbar_quartile=="3"] <- "none"
TTG_data$clustbar_combo <- as.factor(TTG_data$clustbar_combo)
table (TTG_data$clustbar_combo)

color_easy <- c("firebrick1", "pink", "steelblue1", "royalblue3", "ivory3")[TTG_data$clustbar_combo]
plot(TTG_data$strml_barrier, TTG_data$strml_clustering, col=color_easy, pch=16,
     panel.first = grid(col = "ivory4"),
     xlab="Stromal Barrier",
     ylab = "Stromal Clustering",
     cex.lab=1.3, cex.axis=1.2)

```

Figure 3d

```

### KM plot of the 4 groups defined in Fig 3c
time <- TTG_data$times
status <- TTG_data$patient.vital_status

sfit <- survfit(Surv(time, status)~ clustbar_combo, data=TTG_data)

```

```

colorpal = brewer.pal(n=4, name="BrBG")
colorpal = brewer.pal(n=4, name="RdYlBu")
colorpal2 = c("firebrick1", "pink", "steelblue1", "royalblue3")

ggsurv <- ggsurvplot(sfit, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650), break.time.by=1000, legend
  palette = colorpal2,
  ylab= "Overall Survival",
  font.title = c(20, "plain"),
  legend.labs = c ("high clustering, high barrier", "high clustering, low barrier",
  font.x = c(20, "plain"),
  font.y = c(20, "plain"),
  font.tickslab = c(16, "plain"),
  tables.col = "strata",
  tables.y.text = FALSE,
  legend.title = "",
)

ggsurv$plot <- ggsurv$plot +
  theme(legend.text = element_text(size = 14, color = "black"))
ggsurv

### cox proportional hazards test
combo_cox <- coxph(Surv(times, patient.vital_status) ~ clustbar_combo, data=TTG_data)
summary(combo_cox)

```

Figure 3e

```

### lymphocyte% across these 4 groups defined in Fig 3c
boxplot (TTG_data$lym_percent ~ TTG_data$clustbar_combo,
  col= "white",
  border = colorpal2,
  outline=FALSE,
  ylab= "Lymphocyte%", cex.lab=1.5,
  cex.axis = 1.5,
  frame=F)

stripchart(TTG_data$lym_percent ~ TTG_data$clustbar_combo, data = TTG_data,
  vertical = TRUE, method = "jitter",
  pch = 16, col = c("firebrick1", "pink", "steelblue1", "royalblue3") , bg = "black",
  add = TRUE)

### Tukey test to do pairwise comparisons
tt <- TukeyHSD(clustbarcombo_lympho_aov)
tt$`TTG_data$clustbar_combo`[, "p adj"] #this gives full pvalues

```

Figure 4a

```
install.packages("readxl")
library("readxl")

boxplot(ratioLymArea ~ strmbar_quartile2only, data = TTG_data)
boxplot(ratioLymArea ~ strml_clustering_hcateg, data = TTG_data) #1= low clustering, 2= high clustering
```

Figure 4b

```
### compare stromal clustering and barrier across 3 immune phenotypeS described by Tamborero et al
# Immune phenotypeS 1 ('low cytotoxicity'): Cluster 1
# Immune phenotypeS 2 ('intermediate cytotoxicity'): Cluster 2
# Immune phenotypeS 3 ('high cytotoxicity'): Cluster 3

TTG_data$Immune.phenotypeS <- as.factor(TTG_data$Immune.phenotypeS)
kruskal.test(strml_barrier ~ Immune.phenotypeS, data = TTG_data)
kruskal.test(strml_clustering ~ Immune.phenotypeS, data = TTG_data)

### dunn test for groupwise comparison of stromal barrier across 3 immune phenotypes
dunn.test::dunn.test(strml_barrier, Immune.phenotypeS, method = "Hochberg")

### plot strmbar across 3 immune phenotypes
colorpal = c("firebrick4", "firebrick3", "indianred1")
clus <- c("Cluster 1 \n n=216", "Cluster 2 \n n=86", "Cluster 3 \n n=72")
### altering border width to accomodate ticks
par(mar = c(5,5,5,5),
    mgp = c(4, 2.5, 0),
    mai = c(2.0, 1.5, 0.8, 0.4))

boxplot(strml_barrier ~ Immune.phenotypeS, data = TTG_data,
        main="",
        xlab="Immune phenotypes",
        ylab="Stromal barrier",
        outcol= "white",
        border= colorpal,
        cex.lab=1.5,
        cex.axis = 1.5,
        names=c("Cluster 1 \n n=216", "Cluster 2 \n n=86", "Cluster 3 \n n=72"),
        frame= F)

stripchart(strml_barrier ~ Immune.phenotypeS,
           vertical = TRUE,
           data = TTG_data,
           method = "jitter",
           add = TRUE, pch = 16,
           col = colorpal)
```

Figure 4c

```
### multiple wilcoxon (mann whitney) tests for all immune scores: low vs high stromal barrier
wilcoxtests_tombimm <- lapply(
  TTG_data[, 70:85],
  function(x) {wilcox.test(x ~ strmba_quartile2only, data = TTG_data) })
wilcoxtests_tombimm

### multiple wilcoxon (man whitney) tests for all immune scores: low vs high stromal clustering
wilcoxtests_tambimm_clust <- lapply(
  TTG_data[, 70:85],
  function(x) {wilcox.test(x ~ strml_clustering_hcateg, data = TTG_data) })
wilcoxtests_tambimm_clust

### Boxplots
### Treg
boxplot(Treg ~ strmba_quartile2only, data = TTG_data,
  main="",
  ylab="T-reg score",
  ylim = c(-0.7, 0.8),
  xlab = "",
  outcol= "white",
  border= "cyan3",
  cex.lab=1.5,
  cex.axis = 1.5,
  frame=F)

text(x= 1.5, y= 0.5, labels= "p=0.04", cex = 1.5)

stripchart(Treg ~ strmba_quartile2only,
  vertical = TRUE,
  data = TTG_data,
  method = "jitter",
  add = TRUE, pch = 16,
  col = "cyan2")

### Temm
boxplot(Tem ~ strmba_quartile2only, data = TTG_data,
  main="",
  ylab="T-effector memory score",
  ylim = c(-0.7, 0.8),
  xlab = "",
  outcol= "white",
  border= "darkturquoise",
  cex.lab=1.5,
  cex.axis = 1.5,
  frame=F)
text(x= 1.5, y= 0.5, labels= "p=0.003", cex = 1.5)

stripchart(Tem ~ strmba_quartile2only,
  vertical = TRUE,
```

```

data = TTG_data,
method = "jitter",
add = TRUE, pch = 16,
col = "darkturquoise")

### CD8 t cells
boxplot(`CD8+ T` ~ strml_clustering_hcateg, data = TTG_data,
main="",
ylab="CD8 T-cell score",
xlab = "",
outcol= "white",
border= "aquamarine2",
cex.lab=1.5,
cex.axis = 1.5,
frame = F)
text(x= 1.5, y= 0.5, labels= "p=0.03", cex = 1.5)

stripchart(`CD8+ T` ~ strml_clustering_hcateg,
vertical = TRUE,
data = TTG_data,
method = "jitter",
add = TRUE, pch = 16,
col = "aquamarine2")

```