Checkpoint

2024-07-08

Loading, Cleaning, and Scaling Lung Data

```r
lung_meta40 <- read.csv("./lung_meta40.csv")

# Select columns (include proportions and K values)
# new_lung_meta40 <- lung_meta40 |>
#   select(starts_with("k"))

new_lung_meta40 <- lung_meta40 |>
  select(starts_with("k"), starts_with("p")) |>
  select(-c(pack_years, patient_id))

# Scale data and remove rows containing NA's
new_lung_meta40 <- as.data.frame(scale(new_lung_meta40))
new_lung_meta40 <- na.omit(new_lung_meta40)
```
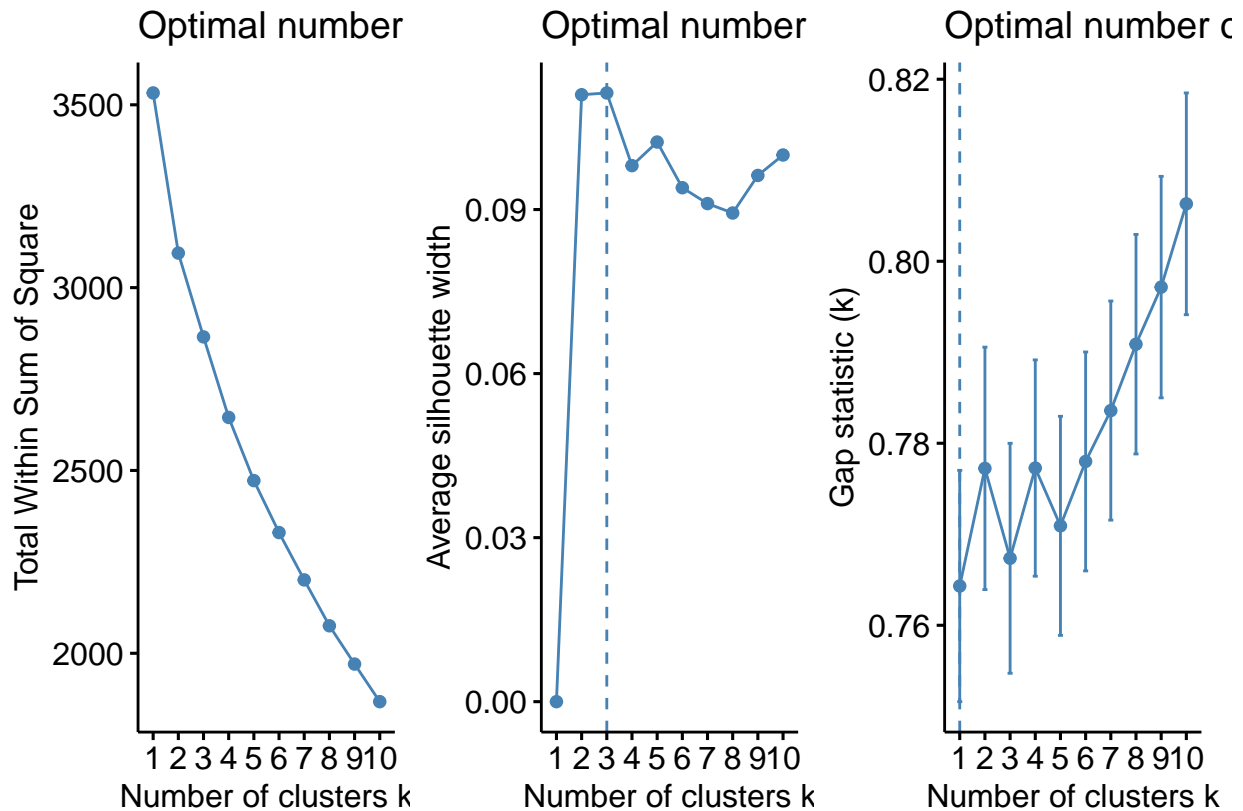
Assessing Number of Clusters

```r
# width sum of squares method (elbow plot)
wss_lung = fviz_nbclust(new_lung_meta40, hcut, method = "wss")

# silhouette method
silhouette_lung = fviz_nbclust(new_lung_meta40, hcut, method = "silhouette")

# gap statistic method
gap_stat_lung = fviz_nbclust(new_lung_meta40, hcut, method = "gap_stat")
wss_lung + silhouette_lung + gap_stat_lung
```

Based on these results, we are planning to use two clusters hierarchical clustering (using Ward distance).

Performing PCA

```
pca_lung40 <- prcomp(new_lung_meta40)

## view cumulative proportion of variance
summary(pca_lung40)
```

```
## Importance of components:
##                            PC1    PC2     PC3    PC4    PC5     PC6     PC7
## Standard deviation      2.4451 1.8538 1.48858 1.3858 1.2394 1.18577 1.04430
## Proportion of Variance  0.2285 0.1313 0.08468 0.0734 0.0587 0.05374 0.04168
## Cumulative Proportion   0.2285 0.3598 0.44449 0.5179 0.5766 0.63033 0.67201
##                             PC8     PC9    PC10    PC11    PC12   PC13    PC14
## Standard deviation      1.00139 0.96219 0.92481 0.86300 0.81536 0.7892 0.75500
## Proportion of Variance  0.03832 0.03538 0.03269 0.02846 0.02541 0.0238 0.02178
## Cumulative Proportion   0.71033 0.74571 0.77840 0.80686 0.83227 0.8561 0.87785
##                            PC15    PC16    PC17    PC18    PC19    PC20   PC21
## Standard deviation      0.67577 0.65598 0.65082 0.59502 0.56540 0.55494 0.4661
## Proportion of Variance  0.01745 0.01645 0.01619 0.01353 0.01222 0.01177 0.0083
## Cumulative Proportion   0.89531 0.91175 0.92794 0.94147 0.95369 0.96546 0.9738
##                            PC22   PC23    PC24    PC25    PC26      PC27
## Standard deviation      0.44829 0.4217 0.34634 0.33677 0.27283 1.343e-15
```

```
## Proportion of Variance 0.00768 0.0068 0.00458 0.00433 0.00284 0.000e+00
## Cumulative Proportion  0.98144 0.9882 0.99282 0.99716 1.00000 1.000e+00
```

We will be using five principal components, which has a cumulative proportion of 0.627.

Performing Hierarchical Clustering

```r
set.seed(42)
# # Perform Hierarchical clustering using Ward distance and first 5 principle components
# ## Produces same dendrogram, but want different objects to draw clusters on
# hier_clust2 <- hcut(pca_lung40$x[,1:5], k = 2)
# ## 3 clusters
# hier_clust3 <- hcut(pca_lung40$x[,1:5], k = 3)
# ## 4 clusters
# hier_clust4 <- hcut(pca_lung40$x[,1:5], k = 4)
#
# # View dendrogram and clusters
# ## 2 clusters
# plot(hier_clust2)
# rect.hclust(hier_clust2, k = 2)
# ## 3 clusters
# plot(hier_clust3)
# rect.hclust(hier_clust3, k = 3)
# ## 4 clusters
# plot(hier_clust4)
# rect.hclust(hier_clust4, k = 4)
# ## silhouette scores for hierarchical clustering
# fviz_silhouette(hier_clust2) # 0.4
# fviz_silhouette(hier_clust3) # 0.31
# fviz_silhouette(hier_clust4) # 0.31

# Perform Hierarchical clustering using Ward distance and first 5 principle components
## Produces same dendrogram, but want different objects to draw clusters on
hier_clust2 <- hcut(new_lung_meta40, k = 2)
## 3 clusters
hier_clust3 <- hcut(new_lung_meta40, k = 3)
## 4 clusters
hier_clust4 <- hcut(new_lung_meta40, k = 4)

# View dendrogram and clusters
## 2 clusters
plot(hier_clust2)
rect.hclust(hier_clust2, k = 2)
```
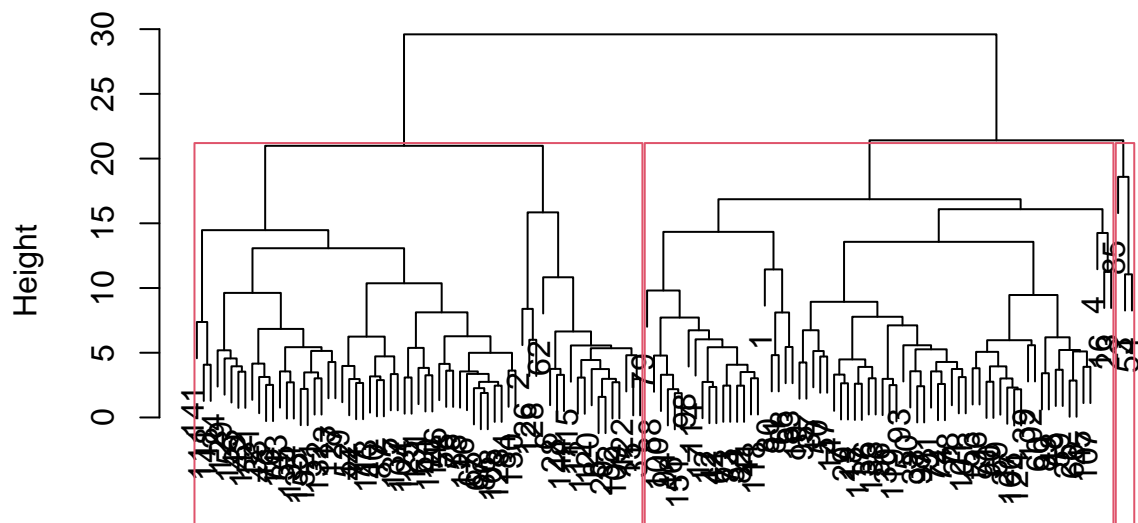
Cluster Dendrogram

x
stats::hclust (*, "ward.D2")
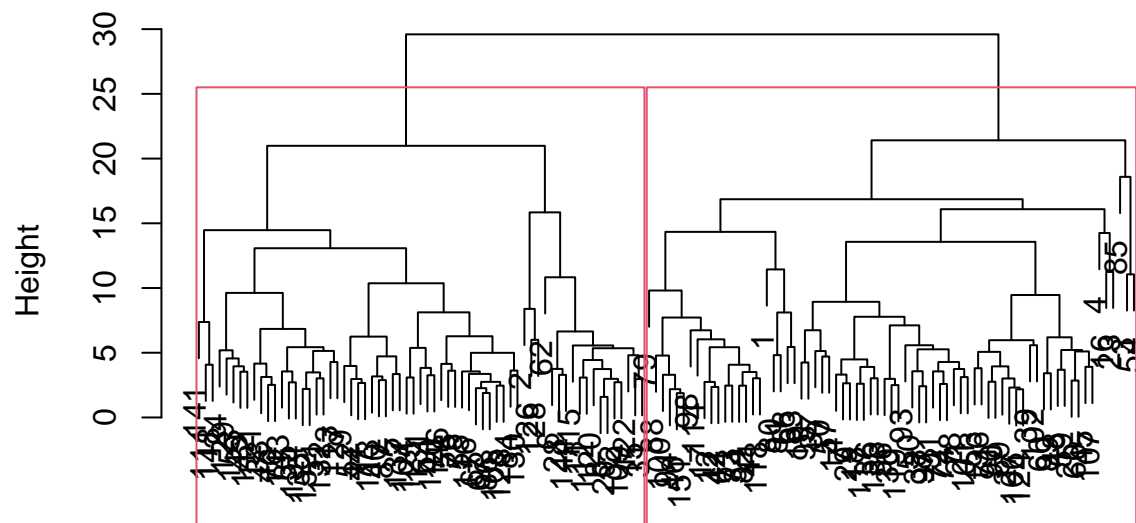
```r
## 3 clusters
plot(hier_clust3)
rect.hclust(hier_clust3, k = 3)
```
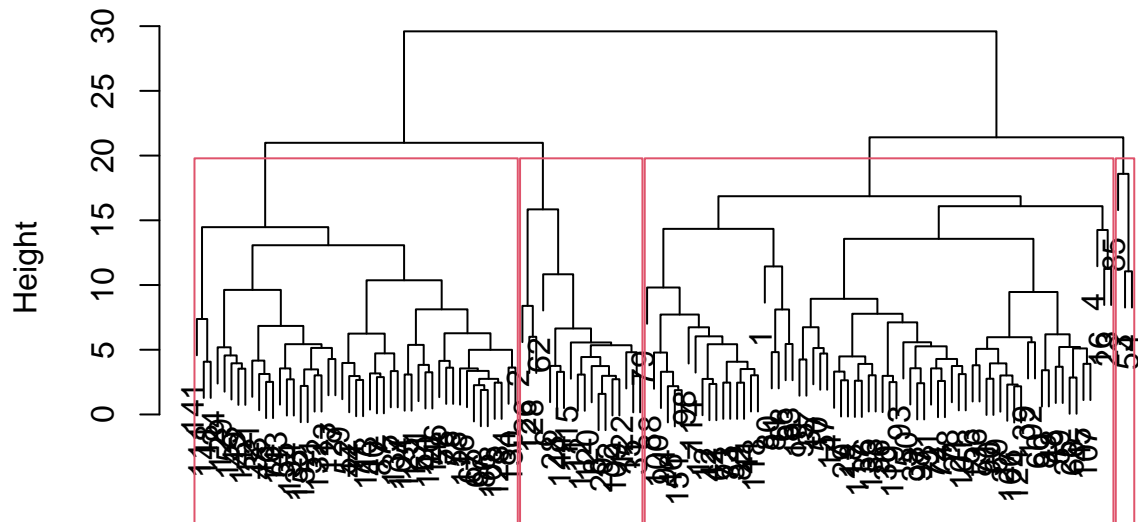
Cluster Dendrogram

x
stats::hclust (*, "ward.D2")

```
## 4 clusters
plot(hier_clust4)
rect.hclust(hier_clust4, k = 4)
```
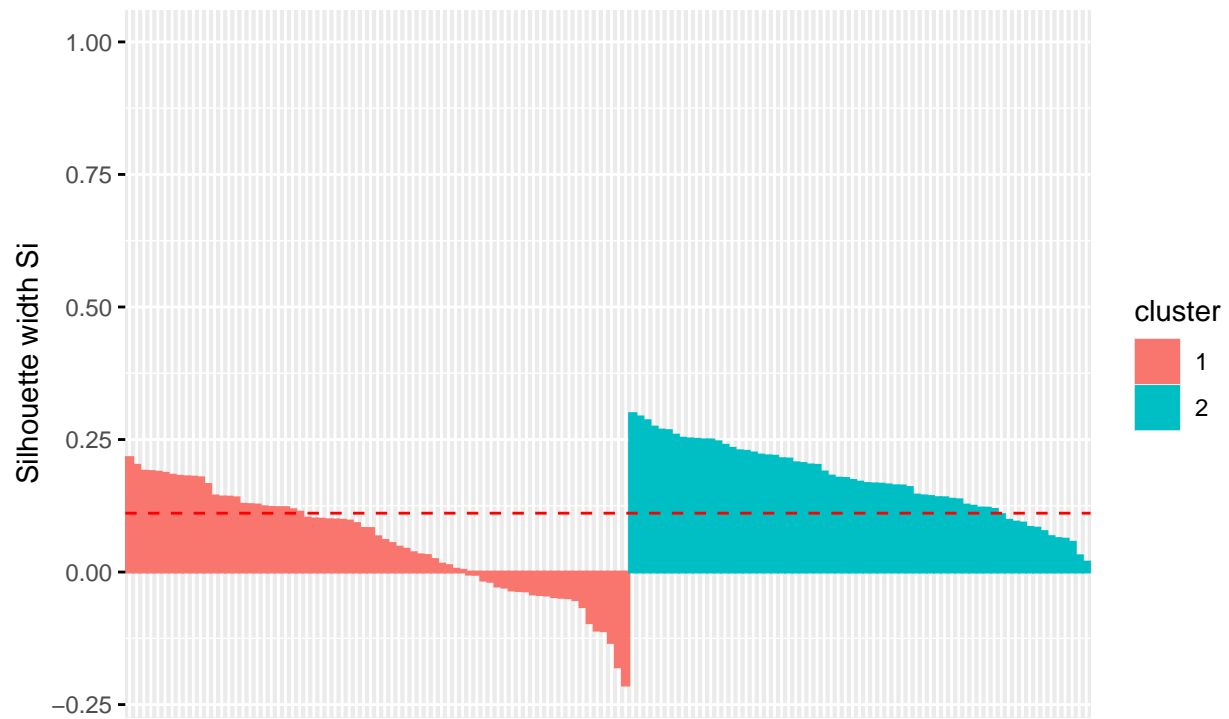
Cluster Dendrogram

x
stats::hclust (*, "ward.D2")

```r
## silhouette scores for hierarchical clustering
fviz_silhouette(hier_clust2) # 0.11
```
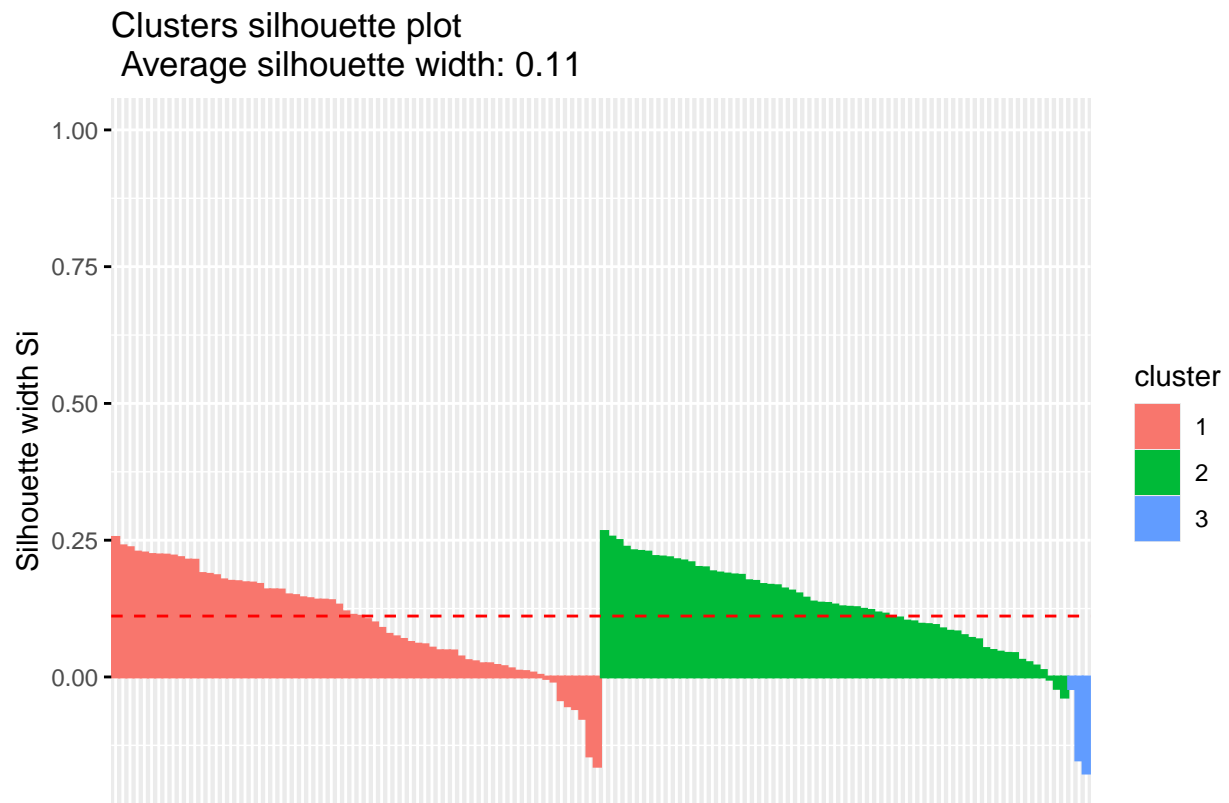
```
##   cluster size ave.sil.width
## 1       1   71          0.05
## 2       2   65          0.17
```

Clusters silhouette plot
Average silhouette width: 0.11
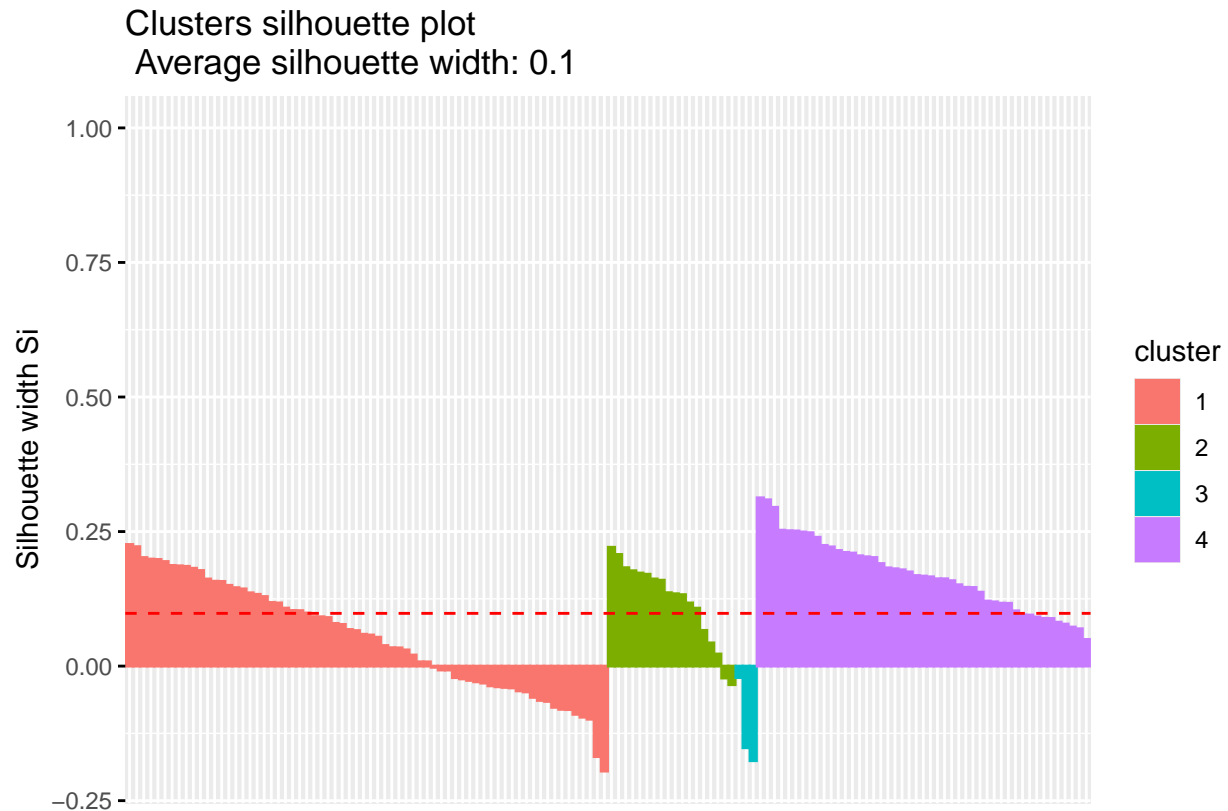


```r
fviz_silhouette(hier_clust3) # 0.11
```

```
##   cluster size ave.sil.width
## 1       1   68          0.10
## 2       2   65          0.13
## 3       3    3         -0.12
```

Clusters silhouette plot
Average silhouette width: 0.11


```
fviz_silhouette(hier_clust4) # 0.10
```

```
##   cluster size ave.sil.width
## 1       1   68          0.05
## 2       2   18          0.12
## 3       3    3         -0.12
## 4       4   47          0.17
```

Clusters silhouette plot
Average silhouette width: 0.1

Initial EDA on Clusters Produced Using Hierarchical and K-Means

```r
# Create data frame for EDA for hierarchical clustering
lung_meta40_hier <- lung_meta40 |>
  filter(!is.na(k_CK) & !is.na(k_CD8) & !is.na(k_CD14) & !is.na(k_Other) & !is.na(k_Other) & !is.na(k_CD
        & !is.na(k_CD4) & !is.na(k_CK_CD8) & !is.na(k_CK_CD14) & !is.na(k_CK_Other) & !is.na(k_CK_CD19
        !is.na(k_CK_CD4) & !is.na(k_CD8_CD14) & !is.na(k_CD8_Other) & !is.na(k_CD8_CD19) & !is.na(k_
        & !is.na(k_CD14_Other) & !is.na(k_CD14_CD19) & !is.na(k_CD14_CD4) & !is.na(k_Other_CD19) &
        !is.na(k_Other_CD4) & !is.na(k_CD19_CD4))

# Add clusters as column
lung_meta40_hier$cluster_id_2 <- hier_clust2$cluster
lung_meta40_hier$cluster_id_3 <- hier_clust3$cluster
lung_meta40_hier$cluster_id_4 <- hier_clust4$cluster

# Count number of patients in each cluster
lung_meta40_hier |>
  group_by(cluster_id_2) |>
  count() # 71 cluster 1, 65 cluster 2
```

```
## # A tibble: 2 x 2
## # Groups:   cluster_id_2 [2]
##   cluster_id_2     n
##          <int> <int>
```

```
## 1              1    71
## 2              2    65
```

```
lung_meta40_hier |>
  group_by(cluster_id_3) |>
  count() # 68 cluster 1, 65 cluster 2, 3 cluster 3
```

```
## # A tibble: 3 x 2
## # Groups:   cluster_id_3 [3]
##   cluster_id_3     n
##          <int> <int>
## 1            1    68
## 2            2    65
## 3            3     3
```

```
lung_meta40_hier |>
  group_by(cluster_id_4) |>
  count() # 68 clutser 1, 18 cluster 2, 3 cluster 3, 47 cluster 4
```

```
## # A tibble: 4 x 2
## # Groups:   cluster_id_4 [4]
##   cluster_id_4     n
##          <int> <int>
## 1            1    68
## 2            2    18
## 3            3     3
## 4            4    47
```

```
# create data frame containing medians for all numeric values in each cluster produced using hierarchic
lung_meta40_hier_2 <- lung_meta40_hier |>
  select(-c(cluster_id_3, cluster_id_4))
data<- lung_meta40_hier_2[, sapply(lung_meta40_hier_2, is.numeric)]
cluster_meds_2 <- data |>
  group_by(cluster_id_2) |>
  summarize(across(everything(), median, na.rm = TRUE))

lung_meta40_hier_3 <- lung_meta40_hier |>
  select(-c(cluster_id_2, cluster_id_4))
data<- lung_meta40_hier_3[, sapply(lung_meta40_hier_3, is.numeric)]
cluster_meds_3 <- data |>
  group_by(as.integer(cluster_id_3)) |>
  summarize(across(everything(), median, na.rm = TRUE))

lung_meta40_hier_4 <- lung_meta40_hier |>
  select(-c(cluster_id_3, cluster_id_2))
data <- lung_meta40_hier_4[, sapply(lung_meta40_hier_4, is.numeric)]
cluster_meds_4 <- data |>
  group_by(as.integer(cluster_id_4)) |>
  summarize(across(everything(), median, na.rm = TRUE))

colnames(lung_meta40_hier) <- c("X", "image_id", "patient_id", "gender", "mhcII_status", "age_at_diagnos
                                "stage_at_diagnosis", "stage_numeric", "pack_years", "survival_days",
```

```
                                "survival_status", "cause_of_death", "adjuvant_therapy",
                                "time_to_recurrence_days", "recurrence_or_lung_ca_death", "total_cell",
                                "p_Tumor", "p_CytotoxicT", "p_Macrophage", "p_Other", "p_BCell", "p_THel
                                "k_Tumor", "k_CytotoxicT", "k_Macrophage", "k_Other", "k_BCell", "k_THel
                                "k_Tumor_CytotoxicT", "k_Tumor_Macrophage", "k_Tumor_Other", "k_Tumor_BC
                                "k_Tumor_THelper", "k_CytotoxicT_Macrophage", "k_CytotoxicT_Other",
                                "k_CytoxicT_BCell", "k_CytotoxicT_THelper", "k_Macrophage_Other",
                                "k_Macrophage_BCell", "k_Macrophage_THelper", "k_Other_BCell",
                                "k_Other_THelper", "k_BCell_THelper", "cluster_id_2", "cluster_id_3",
                                "cluster_id_4")
```

Our next step will be to produce a heatmaps.

Parsing data

```
# Making categorical data numeric for heatmaps
lung_meta40_hier2 <- lung_meta40_hier

lung_meta40_hier2$gender <- factor(lung_meta40_hier2$gender, levels = c("M", "F"), labels = c("1","2"))

lung_meta40_hier2$mhcII_status <- factor(lung_meta40_hier2$mhcII_status, levels = c("low", "high"), labe

lung_meta40_hier2$adjuvant_therapy <- factor(lung_meta40_hier2$adjuvant_therapy, levels = c("No", "Yes")

lung_meta40_hier2$gender = as.integer(lung_meta40_hier2$gender)
lung_meta40_hier2$mhcII_status = as.integer(lung_meta40_hier2$mhcII_status)
lung_meta40_hier2$adjuvant_therapy = as.integer(lung_meta40_hier2$adjuvant_therapy)
```
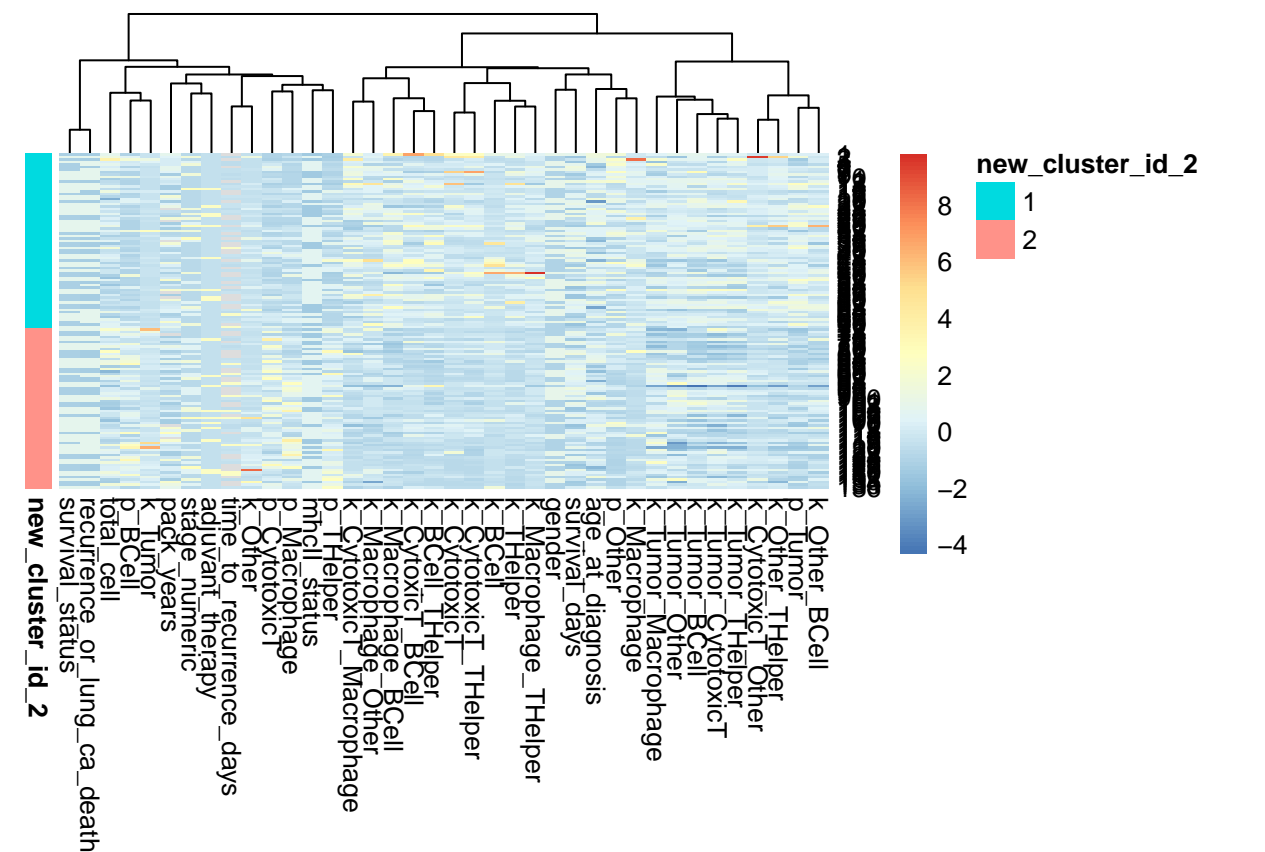
Heatmaps

```
# Creating heatmaps
## 2 clusters
set.seed(42)
file_plot <- lung_meta40_hier2 |>
  select(-c(cluster_id_3, cluster_id_4)) |>
  arrange(cluster_id_2) |>
  mutate(new_cluster_id_2 = factor(cluster_id_2)) |>
  select(-cluster_id_2)
clust <- file_plot |> select(new_cluster_id_2)
rownames(clust) = rownames(file_plot)
heat_df <- file_plot[, sapply(file_plot, is.numeric)] |>
  scale() |>
  as.data.frame() |>
  select(-X) |>
  as.matrix()
rownames(heat_df) = rownames(clust)
rownames(clust)
pheatmap(heat_df,
        annotation_row = clust,
```

```
    cluster_cols = TRUE,
    cluster_rows = FALSE,
    clustering_method = "ward.D2")
```



```
## 3 clusters
file_plot <- lung_meta40_hier2 |>
  select(-c(cluster_id_2, cluster_id_4)) |>
  arrange(cluster_id_3) |>
  mutate(new_cluster_id_3 = factor(cluster_id_3)) |>
  select(-cluster_id_3)
clust <- file_plot |> select(new_cluster_id_3)
rownames(clust) = rownames(file_plot)
heat_df <- file_plot[, sapply(file_plot, is.numeric)] |>
  scale() |>
  as.data.frame() |>
  select(-X) |>
  as.matrix()
rownames(heat_df) = rownames(clust)
rownames(clust)
pheatmap(heat_df,
         annotation_row = clust,
         cluster_cols = TRUE,
         cluster_rows = FALSE,
         clustering_method = "ward.D2")
```

```
## 4 clusters
file_plot <- lung_meta40_hier2 |>
  select(starts_with("k"), starts_with("p"), -pack_years, cluster_id_4) |>
  arrange(cluster_id_4) |>
  mutate(cluster_id = factor(cluster_id_4)) |>
  select(-cluster_id_4)
clust <- file_plot |> select(cluster_id)
rownames(clust) = rownames(file_plot)
heat_df <- file_plot[, sapply(file_plot, is.numeric)] |>
  scale() |>
  as.data.frame() |>
  as.matrix()
rownames(heat_df) = rownames(clust)
rownames(clust)
pheatmap(heat_df,
         annotation_row = clust,
         cluster_cols = TRUE,
         cluster_rows = FALSE,
         clustering_method = "ward.D2")
```
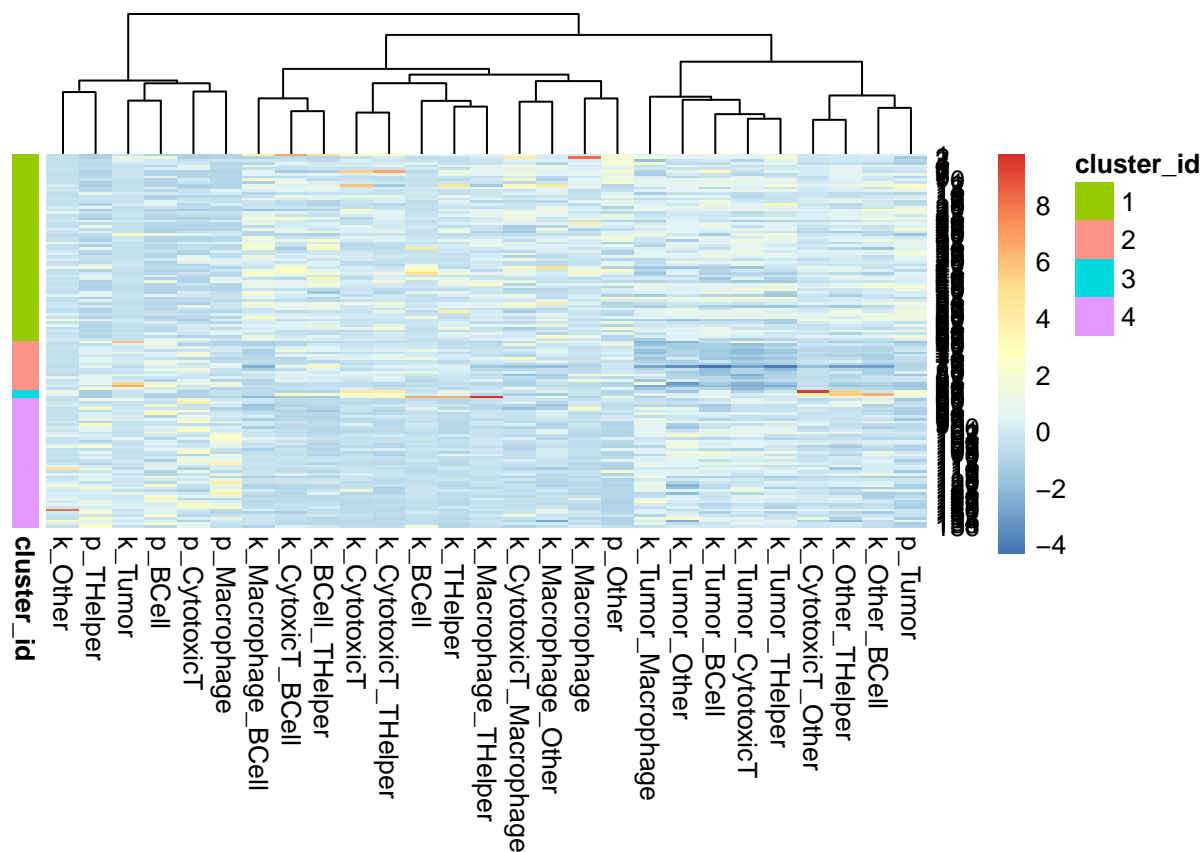
Boxplots for Cluster EDA

```
current_data <- lung_meta40_hier

current_data <- current_data[, sapply(current_data, is.numeric)] |>
  select(-X, -stage_numeric, -survival_status, -recurrence_or_lung_ca_death) |>
  ## Change the cluster id if needed
  pivot_longer(cols = -cluster_id_4,
               names_to = "type",
               values_to = "value") |>
  filter(cluster_id_4 != 3)

current_data |>
  ggplot(aes(x = type, y = value, col = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip() #This is for all, but you can't really tell much
```

```
## Warning: Removed 59 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```
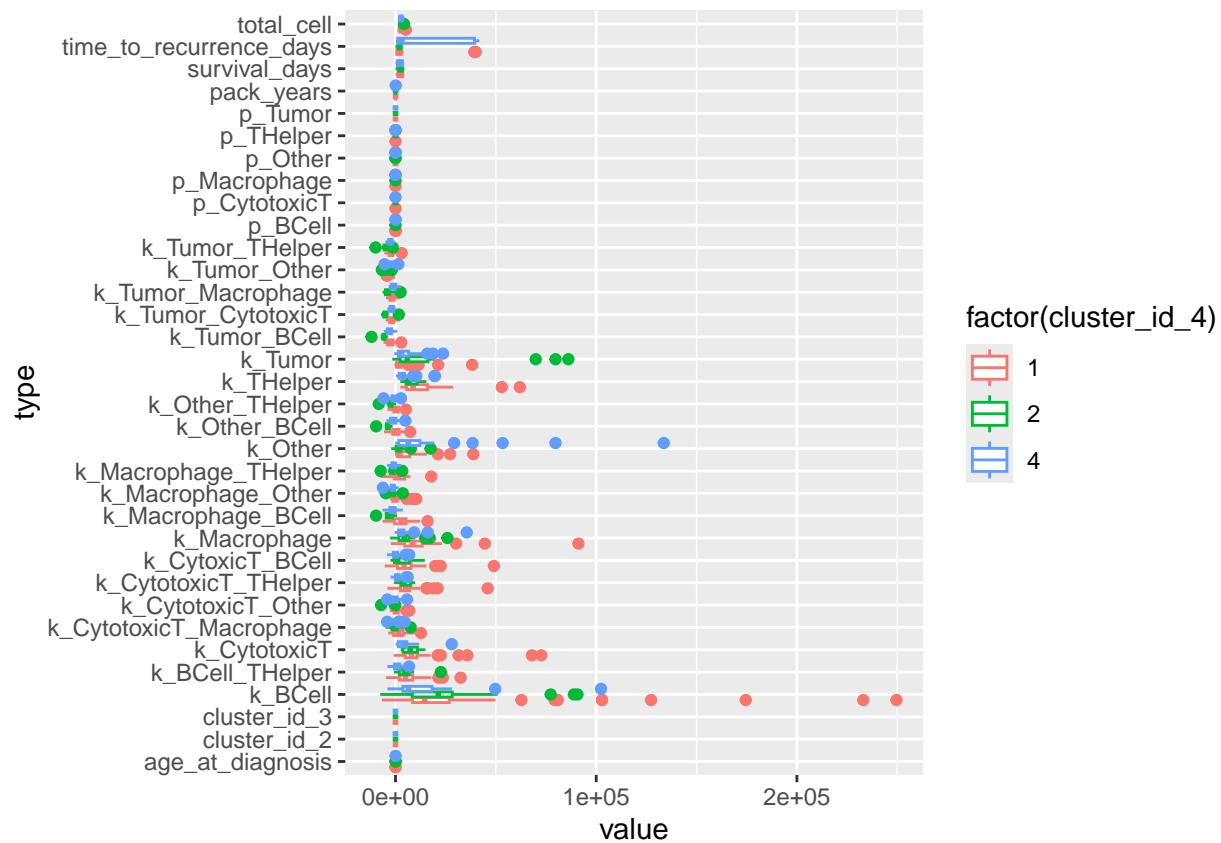
```
## Just Proportions

current_data |>
  filter(startsWith(type, "p_")) |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```
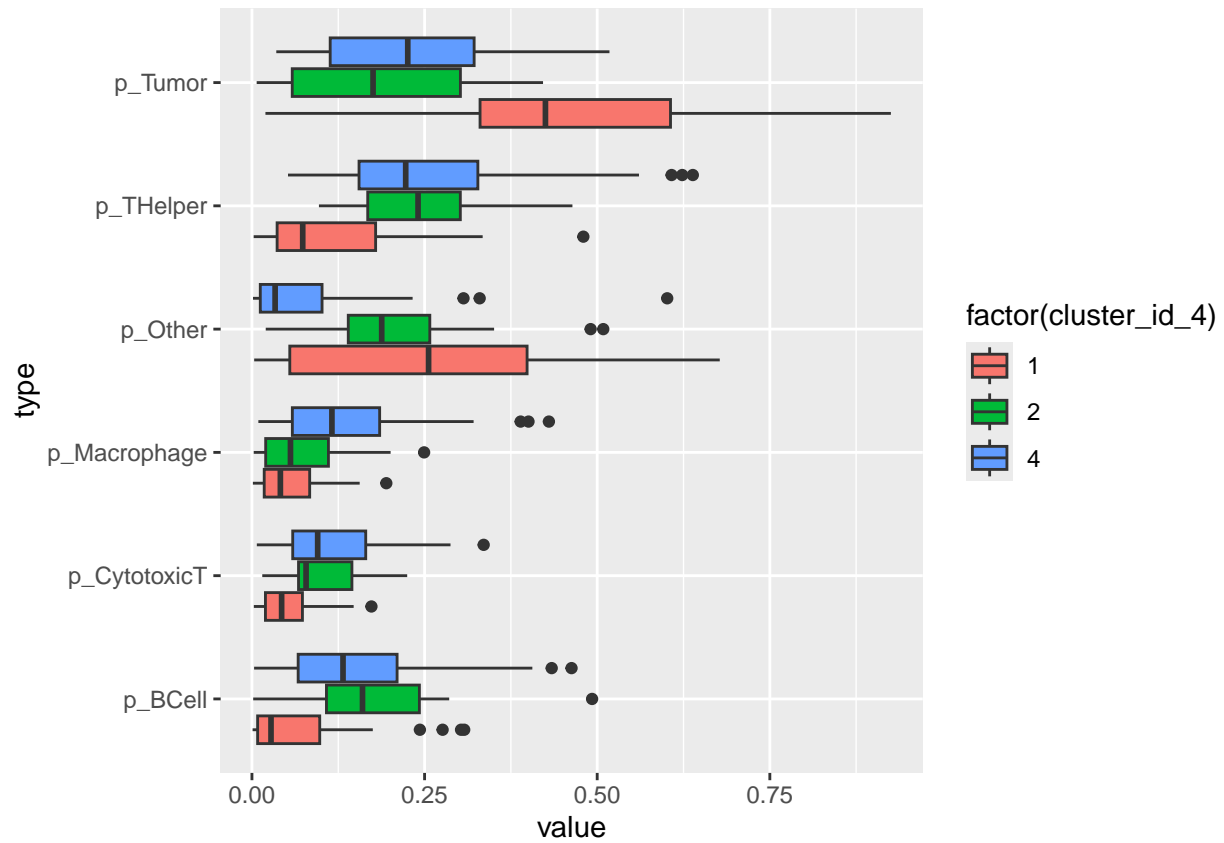
```
## Just K standalone (except CK, because it was harder to see)

current_data |>
  filter(type %in% c('k_CD8', 'k_CD4', 'k_CD19', 'k_CD14', 'k_Other')) |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```
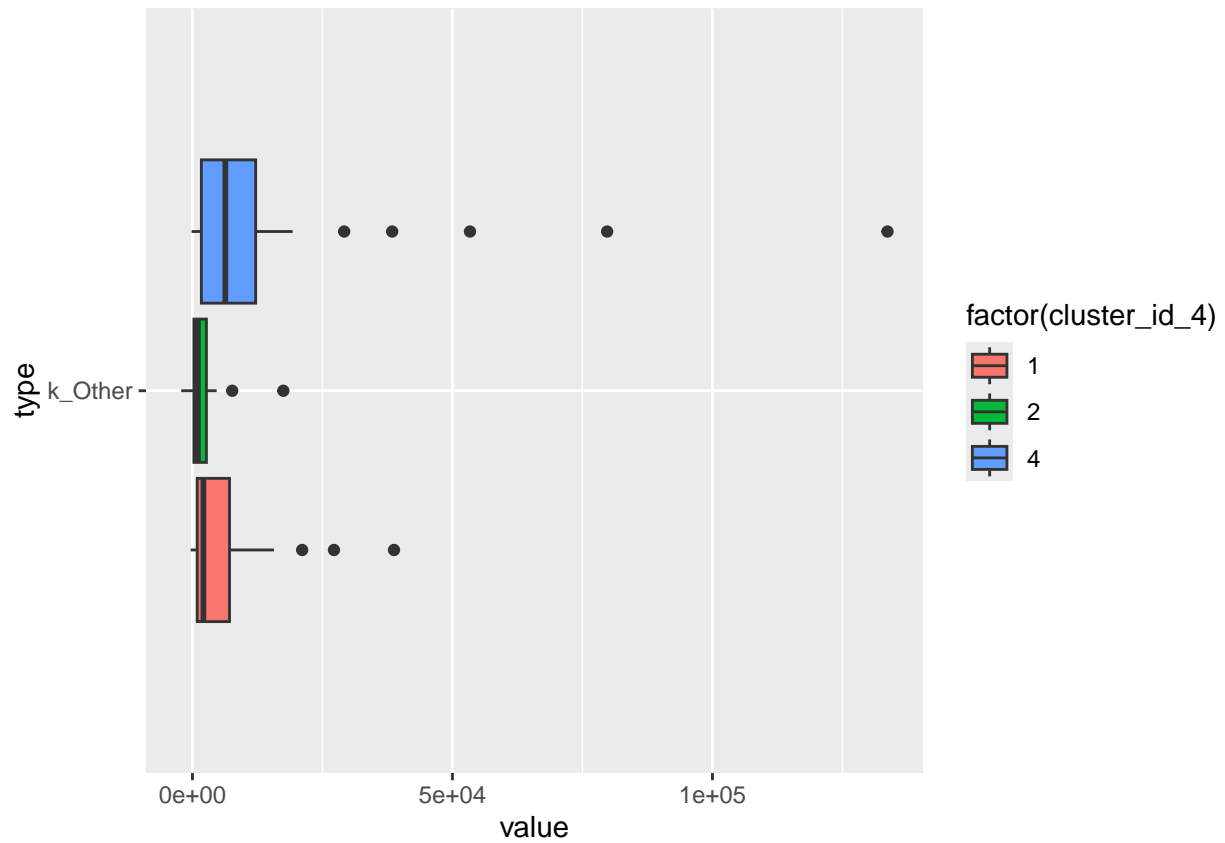
```
current_data |>
  filter(type == 'k_CK') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot()
```

```
## K Crosses for CK (WE ARE USING THIS)

current_data |>
  filter(startsWith(type, "k_CK_")) |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```
## K Crosses for CD8

current_data |>
  filter(startsWith(type, "k_CD8_") | endsWith(type, "_CD8"),
         type != 'p_CD8',
         type != 'k_CD8') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```
# K Crosses for CD14

current_data |>
  filter(startsWith(type, "k_CD14_")  | endsWith(type, "_CD14"),
         type != 'p_CD14',
         type != 'k_CD14') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```
# K Crosses for Other

current_data |>
  filter(startsWith(type, "k_Other_")  | endsWith(type, "_Other"),
         type != 'p_Other',
         type != 'k_Other') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```
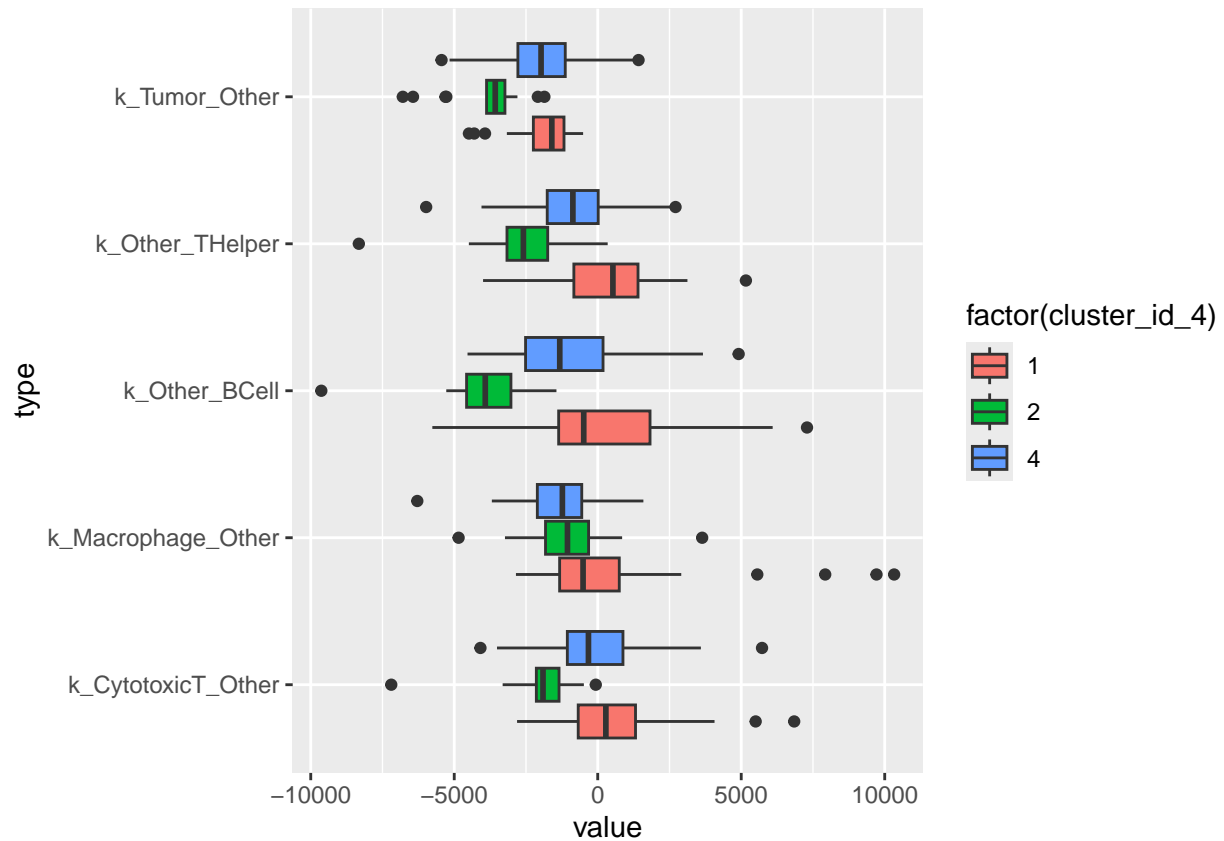
```
# Last K Cross for CD19

current_data |>
  filter(startsWith(type, "k_CD19_")  | endsWith(type, "_CD19"),
         type != 'p_CD19',
         type != 'k_CD19') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```r
# For metadata

current_data |>
  filter(!startsWith(type, "k_"), !startsWith(type, "p_"),
         type != "pack_years", type != 'age_at_diagnosis',
         type != 'cluster_id_3', type != 'cluster_id_2') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```
## Warning: Removed 51 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```
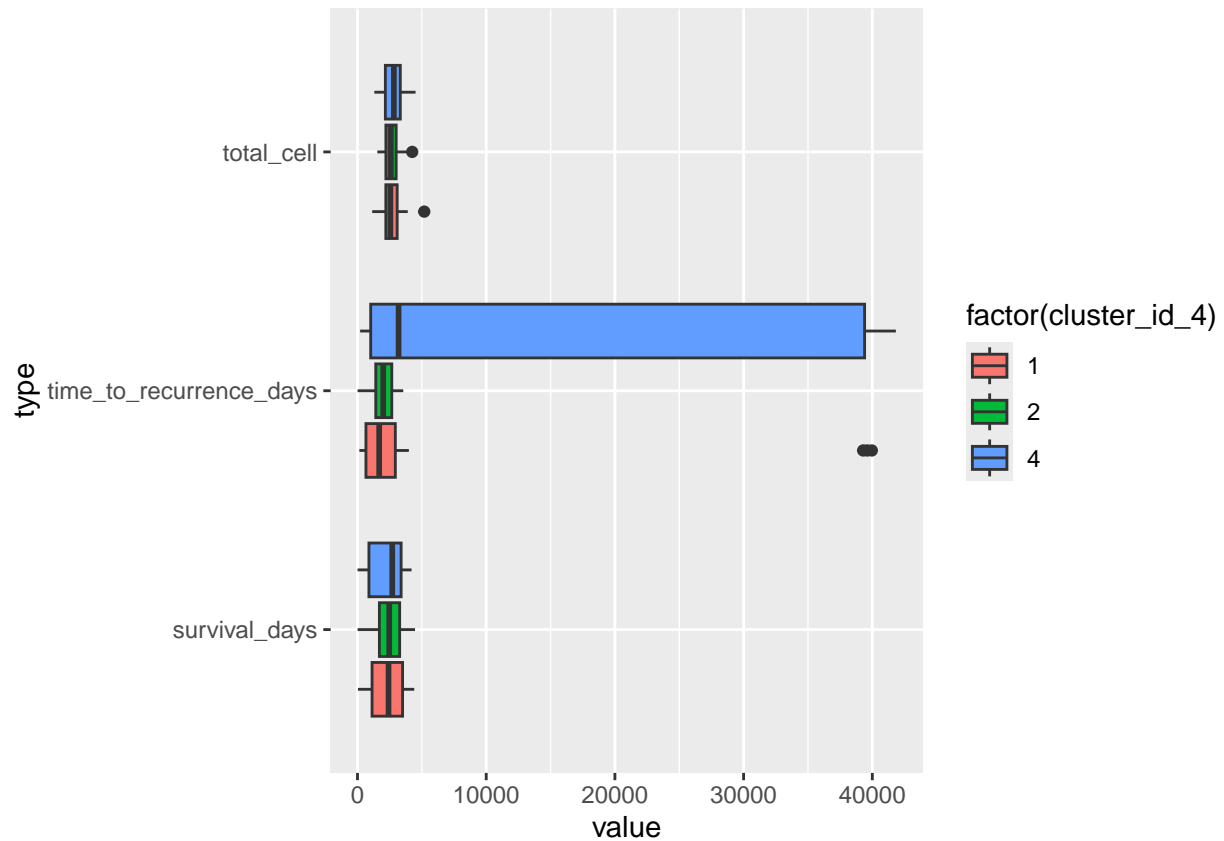
```
current_data |>
  filter(type == "pack_years"| type == 'age_at_diagnosis') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip()
```

```
## Warning: Removed 8 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```
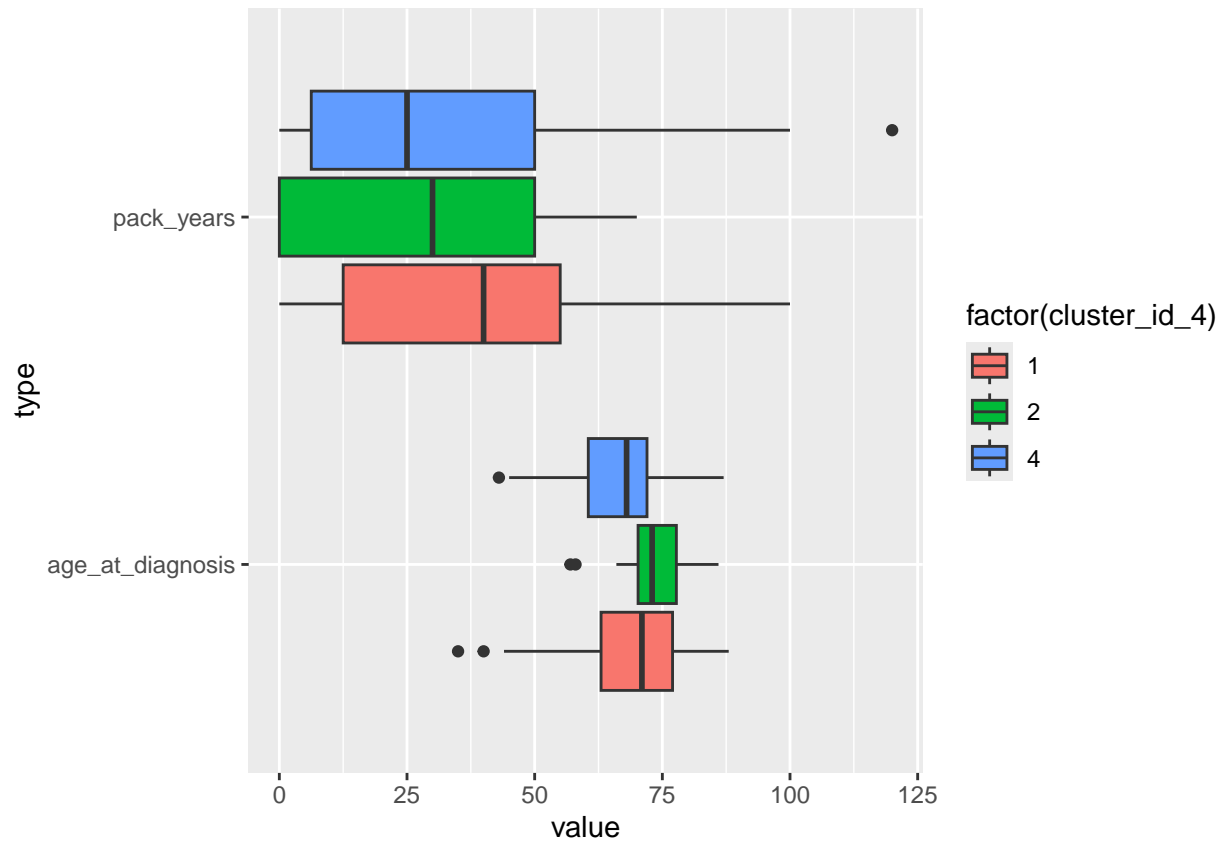
```r
# pack years alone
current_data %>%
  filter(type == "pack_years") %>%
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  scale_fill_discrete(name = "cluster_id") +
  ggtitle("Distribution of Pack Years") +
  labs(y = "Pack Years", x = " ") + theme_bw()
```

```
## Warning: Removed 8 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```

Distribution of Pack Years
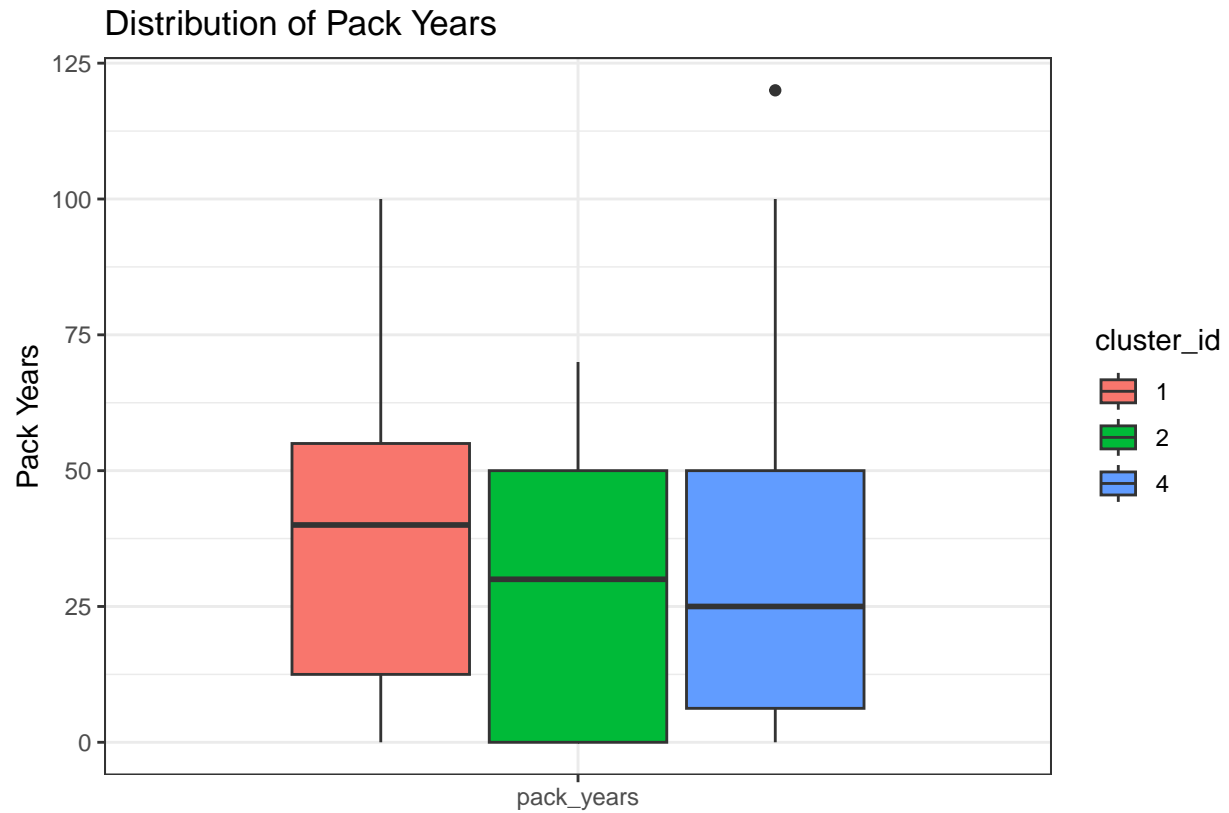

```
# age alone
current_data %>%
  filter(type == "age_at_diagnosis") %>%
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  scale_fill_discrete(name = "cluster_id") +
  ggtitle("Distribution of Age at Diagnosis") +
  labs(y = "Age", x = " ") + theme_bw()
```

Distribution of Age at Diagnosis
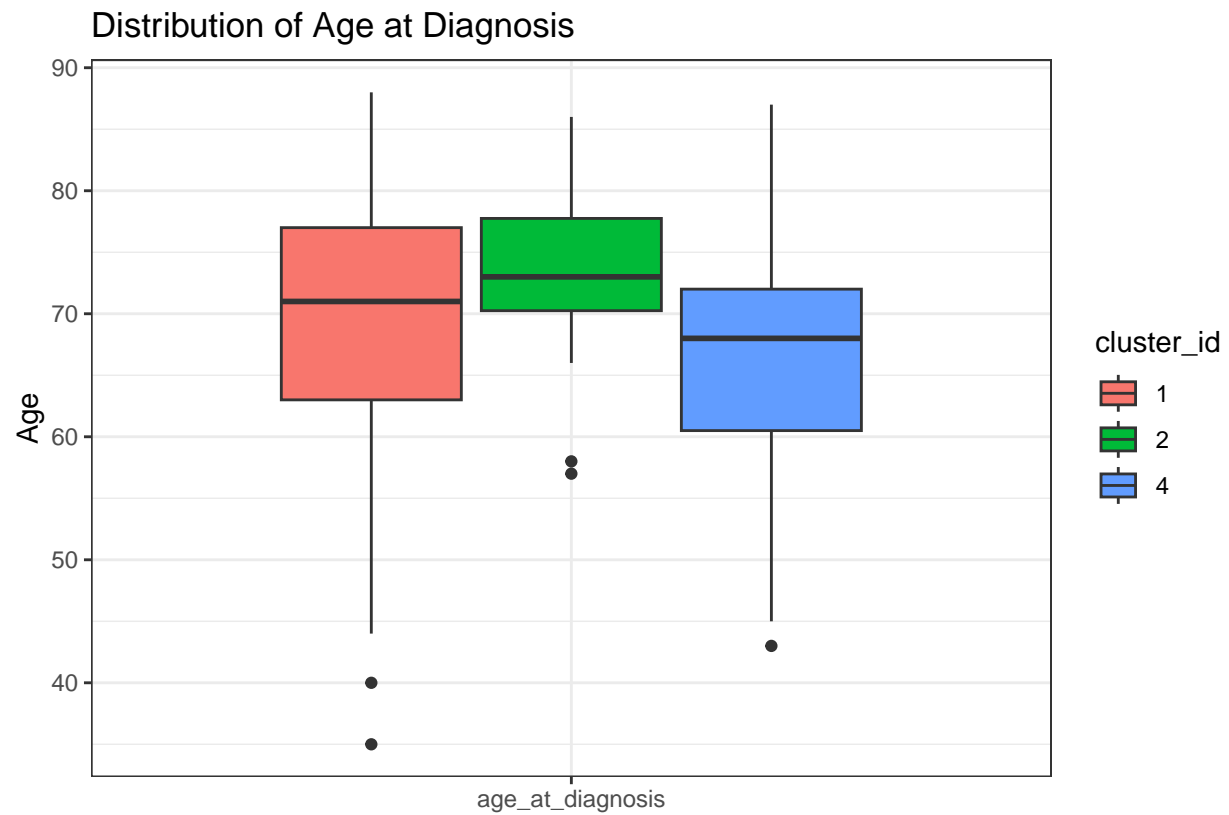
Survival Analysis

```r
current_data <- lung_meta40_hier |>
  filter(cluster_id_4 != 3)

km_fit <- survfit(Surv(survival_days, survival_status) ~ cluster_id_4, data = current_data)
ggsurvplot(km_fit, data = current_data)
```
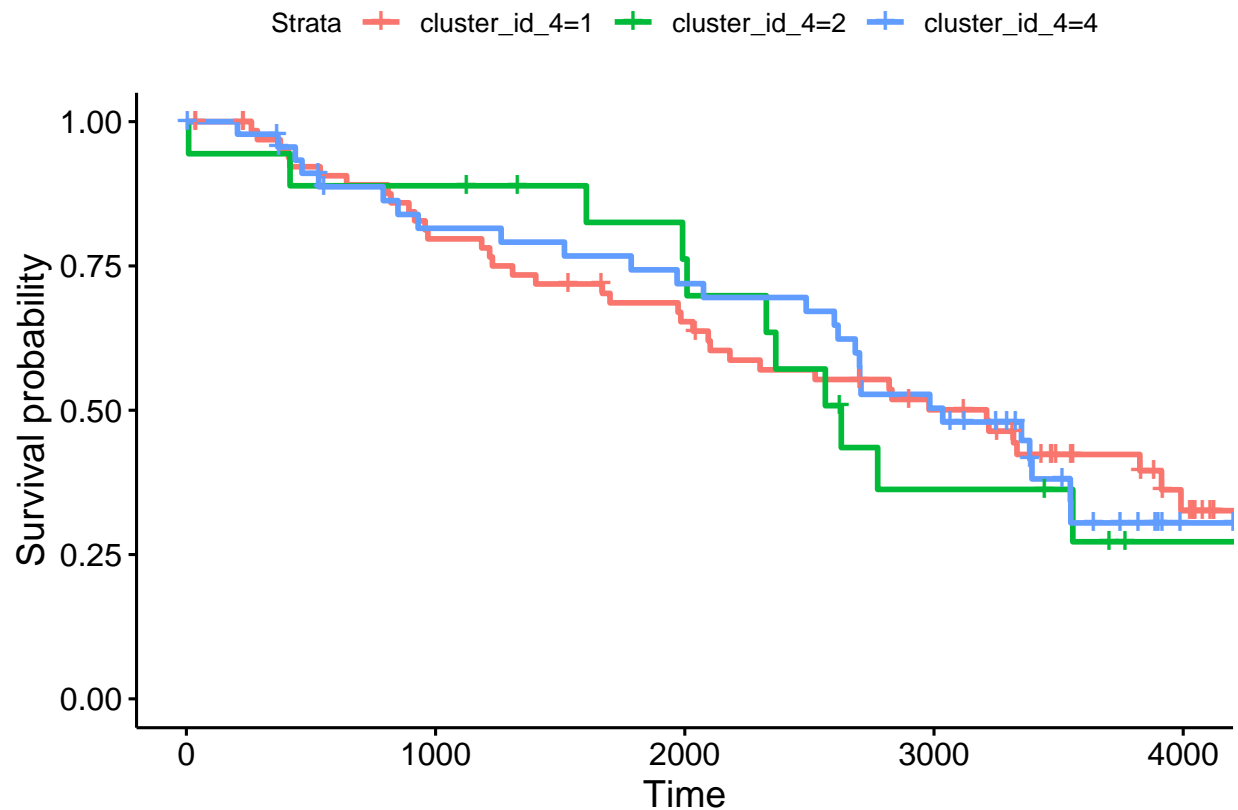
```r
survdiff(Surv(survival_days, survival_status) ~ cluster_id_4, data= current_data, rho = 1)
```

```
## Call:
## survdiff(formula = Surv(survival_days, survival_status) ~ cluster_id_4,
##     data = current_data, rho = 1)
##
##                 N Observed Expected (O-E)^2/E (O-E)^2/V
## cluster_id_4=1 68    26.96    26.75   0.00171   0.00474
## cluster_id_4=2 18     7.42     7.09   0.01588   0.02435
## cluster_id_4=4 47    18.05    18.60   0.01624   0.03407
##
##  Chisq= 0  on 2 degrees of freedom, p= 1
```

Barplots

```r
current_data <- lung_meta40_hier |>
  filter(cluster_id_4 != 3)

current_data <- current_data |> select_if(~!is.numeric(.)) |>
  mutate(stage_numeric = current_data$stage_numeric,
         #Change based on which cluster
         cluster_id = current_data$cluster_id_4) |>
  select(-image_id, -patient_id, -cause_of_death, -stage_at_diagnosis)
```
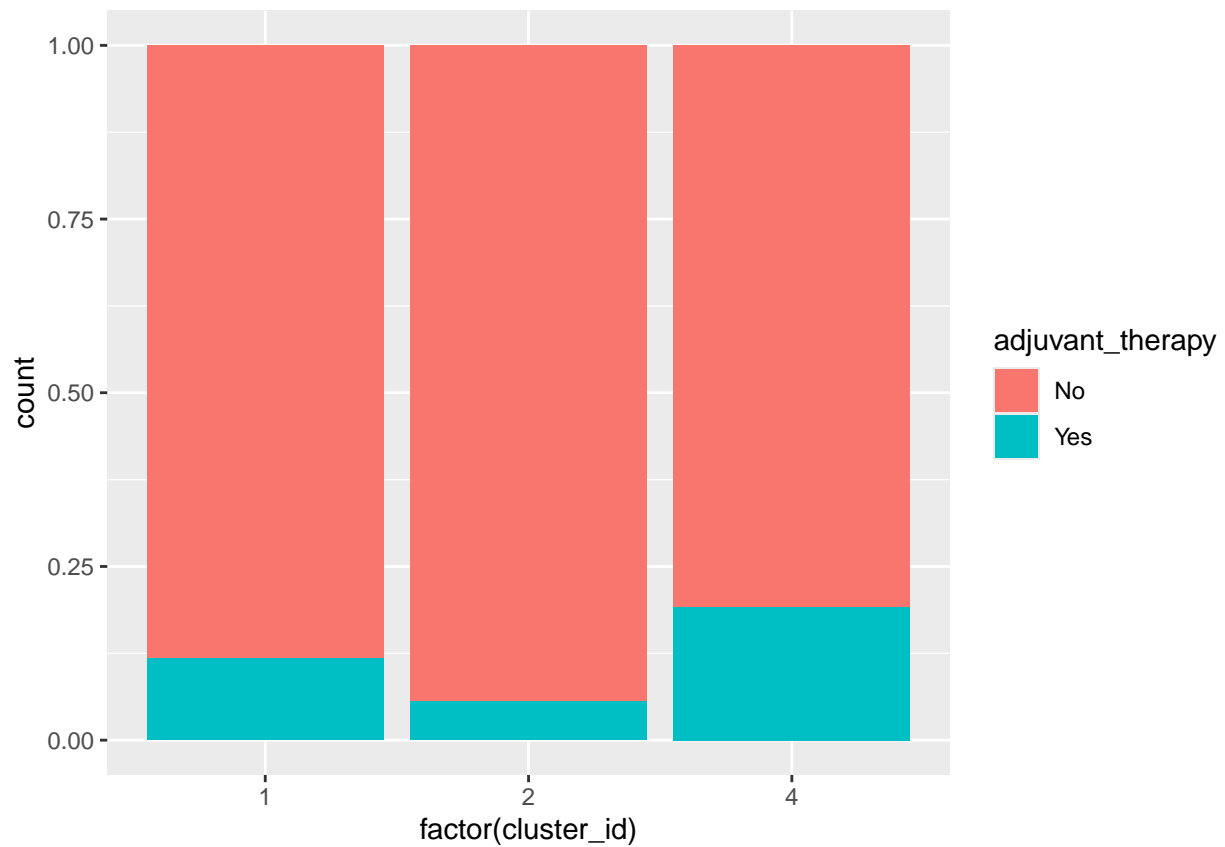
```
current_data |>
  select(cluster_id, gender) |>
  ggplot(aes(x = factor(cluster_id), fill = gender)) +
  geom_bar(position = 'fill') +
  scale_fill_discrete(name = "Gender") +
  ggtitle("Proportions of Gender") +
  labs(y = "Percentage", x = "cluster_id") + theme_bw()
```


Proportions of Gender
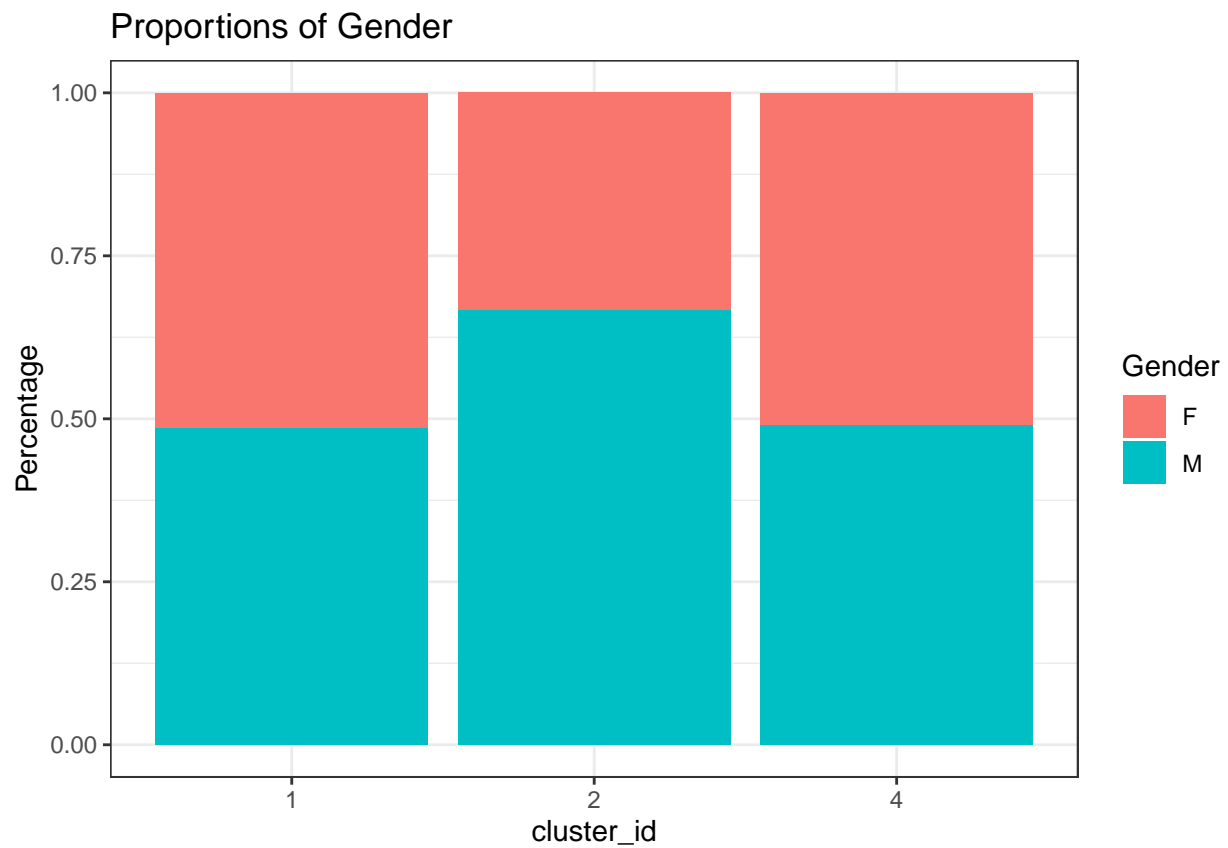
```
current_data |>
  select(cluster_id, mhcII_status) |>
  ggplot(aes(x = factor(cluster_id), fill = mhcII_status)) +
  geom_bar(position = 'fill')
```
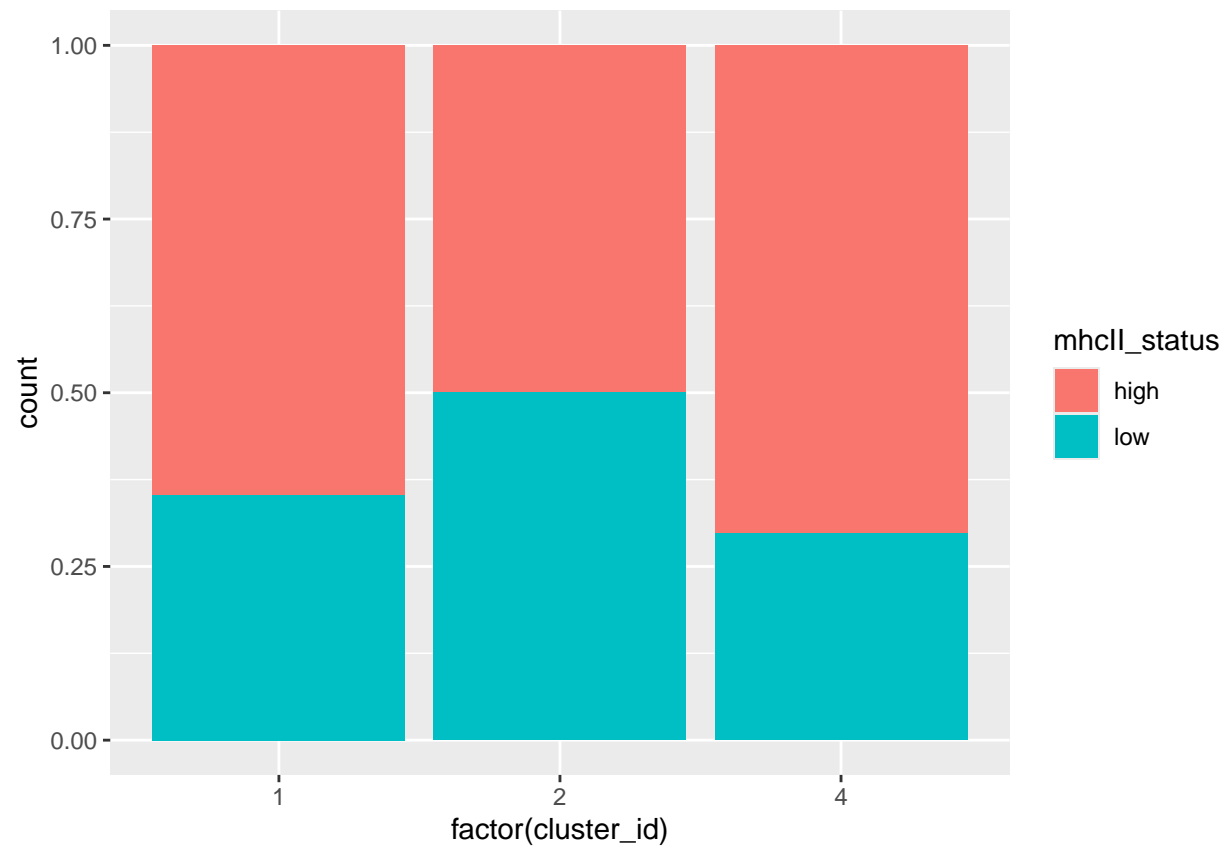
```
current_data |>
  select(cluster_id, adjuvant_therapy) |>
  ggplot(aes(x = factor(cluster_id), fill = adjuvant_therapy)) +
  geom_bar(position = 'fill')
```

```
current_data |>
  select(cluster_id, stage_numeric) |>
  ggplot(aes(x = factor(cluster_id), fill = factor(stage_numeric))) +
  geom_bar(position = 'fill') +
  scale_fill_discrete(name = "Stage of Cancer") +
  ggtitle("Proportions of Stage of Cancer") +
  labs(y = "Percentage", x = "cluster_id") + theme_bw()
```

Proportions of Stage of Cancer
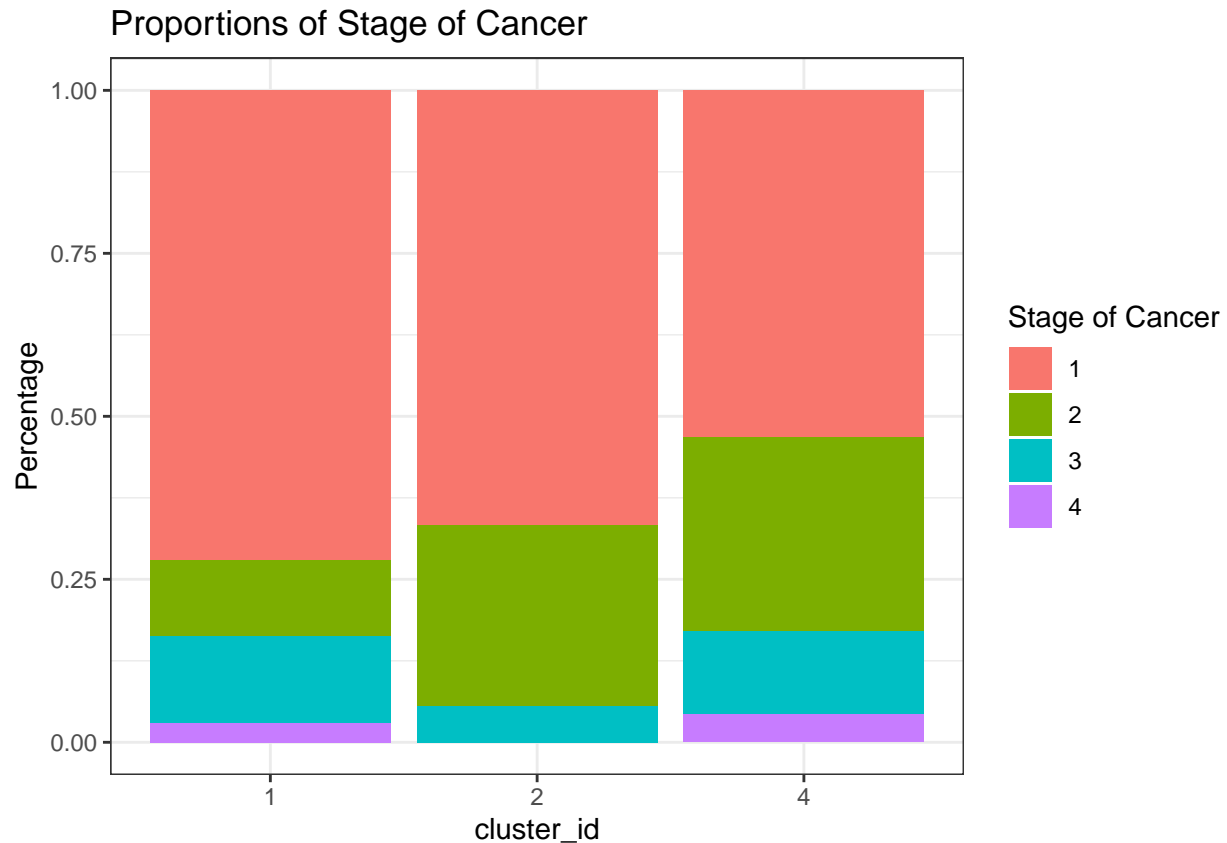
Survival with Time to Recurrence

```
recurrence_data <- lung_meta40_hier |>
  select(patient_id, survival_days, time_to_recurrence_days, cluster_id_4) |>
  mutate(recurrence = if_else(is.na(time_to_recurrence_days), 0, 1),
         recurrence_days = if_else(is.na(time_to_recurrence_days), survival_days, time_to_recurrence_da
  filter(cluster_id_4 != 3)

km_fit_rec <- survfit(Surv(recurrence_days, recurrence) ~ cluster_id_4, data = recurrence_data)
ggsurv <- ggsurvplot(km_fit_rec, data = recurrence_data, xlim = c(0,8500),
                     ylab = 'Non-recurrence Probability')
ggsurv$plot +
  scale_x_continuous(breaks = seq(0, 10000, by = 1000)) +
  labs(title = 'Kaplan-Meier Curve for Cancer Recurrence') +
  theme(plot.title = element_text(hjust = 0.5))
```
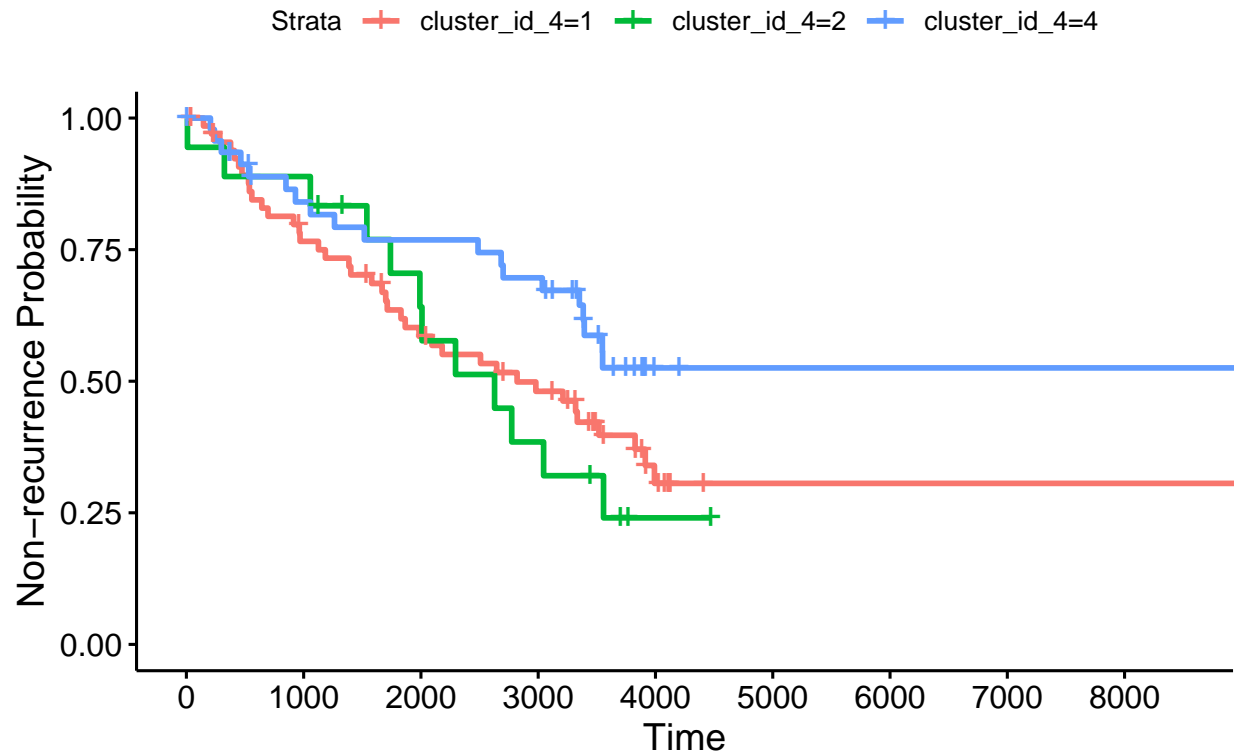
```
## Scale for x is already present.
## Adding another scale for x, which will replace the existing scale.
```

Kaplan–Meier Curve for Cancer Recurrence


```r
survdiff(Surv(recurrence_days, recurrence) ~ cluster_id_4, data = recurrence_data, rho = 1)
```

```
## Call:
## survdiff(formula = Surv(recurrence_days, recurrence) ~ cluster_id_4,
##     data = recurrence_data, rho = 1)
##
##                 N Observed Expected (O-E)^2/E (O-E)^2/V
## cluster_id_4=1 68     29.2    24.49     0.907     2.324
## cluster_id_4=2 18      8.3     6.56     0.464     0.689
## cluster_id_4=4 47     15.0    21.50     1.939     4.646
##
##  Chisq= 4.7  on 2 degrees of freedom, p= 0.1
```

```r
autoplot(km_fit_rec, xlim = c(0, 5000))
```

```
## Warning: Removed 12 rows containing missing values or values outside the scale range
## ('geom_step()').
```
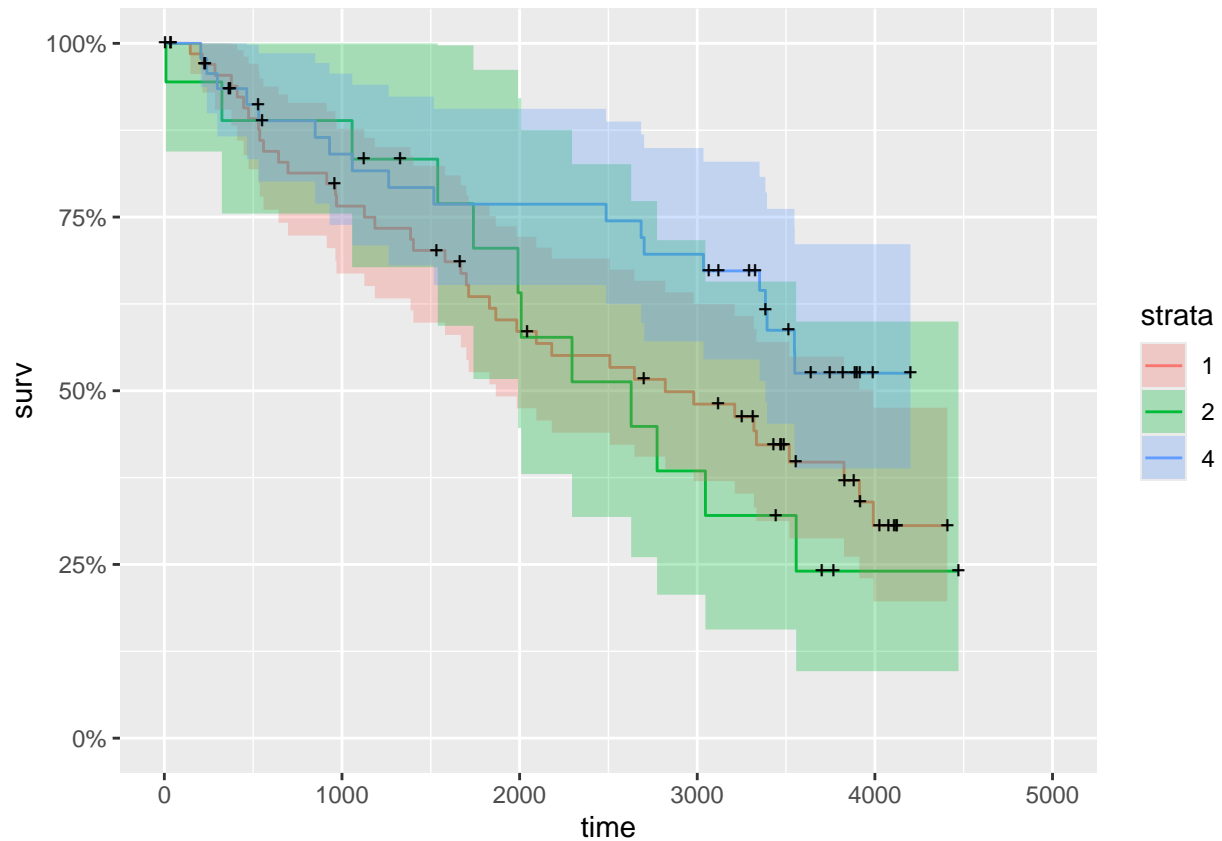
```
recurrence_data |>
  group_by(cluster_id_4) |>
  summarize(p_recurrence = sum(recurrence == 1) / n())
```

```
## # A tibble: 3 x 2
##   cluster_id_4 p_recurrence
##          <int>        <dbl>
## 1            1        0.618
## 2            2        0.667
## 3            4        0.596
```

Boxplots we end up using

```
current_data <- lung_meta40_hier

current_data <- current_data[, sapply(current_data, is.numeric)] |>
  select(-X, -stage_numeric, -survival_status, -recurrence_or_lung_ca_death) |>
  ## Change the cluster id if needed
  pivot_longer(cols = -cluster_id_4,
               names_to = "type",
               values_to = "value") |>
  filter(cluster_id_4 != 3)
```
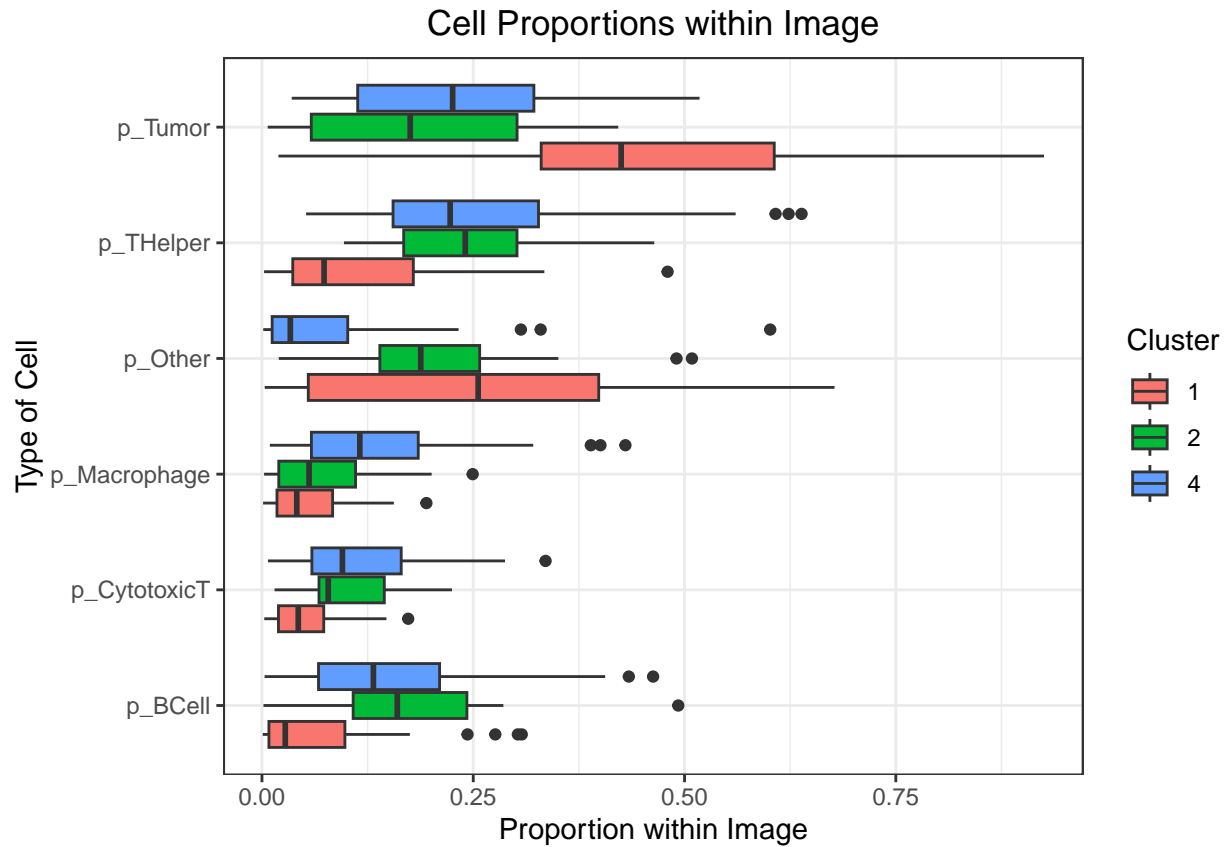
```
current_data |>
  filter(startsWith(type, "k_CK_")) |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip() +
  theme(plot.title = element_text(hjust = 0.5)) +
  theme_bw() +
  labs(title = "Observed K Cross - Permuted K Cross for CK cell interactions",
       x = 'Cell Interaction',
       y = 'Difference',
       fill = 'Cluster')
```

Observed K Cross – Permuted K Cross for CK cell interactions
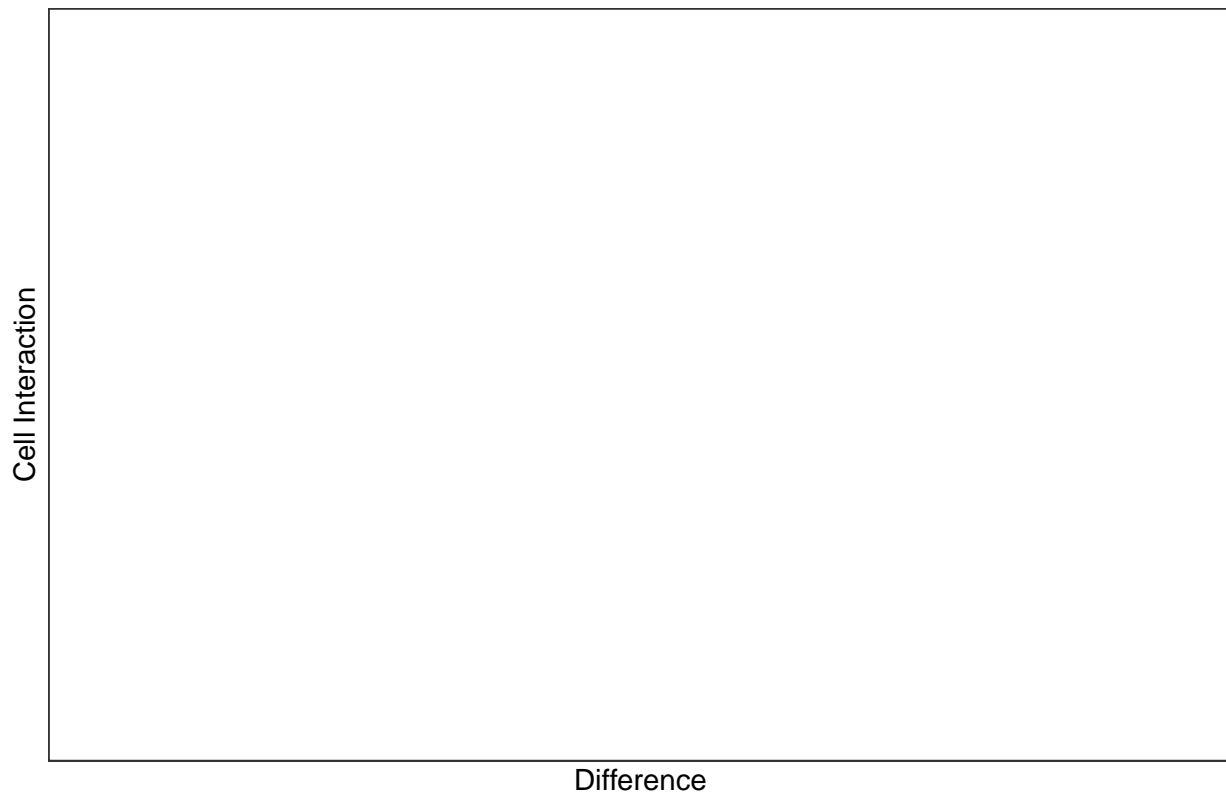
Difference

```
current_data |>
  filter(startsWith(type, "p_")) |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip() +
  theme_bw() +
  labs(title = "Cell Proportions within Image",
       x = 'Type of Cell',
       y = 'Proportion within Image',
       fill = 'Cluster') +
  theme(plot.title = element_text(hjust = 0.5))
```

Cell Proportions within Image

```
current_data |>
  filter(startsWith(type, "k_CD8_") | endsWith(type, "_CD8"),
         type != 'p_CD8',
         type != 'k_CD8') |>
  ggplot(aes(x = type, y = value, fill = factor(cluster_id_4))) +
  geom_boxplot() +
  coord_flip() +
  theme_bw() +
  labs(title = "Observed K Cross - Permuted K Cross for CD8 cell interactions",
       x = 'Cell Interaction',
       y = 'Difference',
       fill = 'Cluster') +
  theme(plot.title = element_text(hjust = 0.5))
```

Observed K Cross – Permuted K Cross for CD8 cell interactions

Cell Interaction

Difference