1 Results

Some more guidelines from the School of Geosciences.

This section should summarize the findings of the research referring to all figures, tables and statistical results (some of which may be placed in appendices).

- include the primary results, ordered logically - it is often useful to follow the same order as presented in the methods.
- alternatively, you may find that ordering the results from the most important to the least important works better for your project.
- data should only be presented in the main text once, either in tables or figures; if presented in figures, data can be tabulated in appendices and referred to at the appropriate point in the main text.

Often, it is recommended that you write the results section first, so that you can write the methods that are appropriate to describe the results presented. Then you can write the discussion next, then the introduction which includes the relevant literature for the scientific story that you are telling and finally the conclusions and abstract – this approach is called writing backwards.

1.1 Determination of inflammatory groups representing intratumor inflammatory status

Functional clusters of osteosarcoma samples were created using k-means clustering of from MDS visualization of the Hallmark Inflammatory Response signature.

Inflammatory groups characterizing the intensity of inflammatory status in tumors were created by first creating groups of inflammatory status. The number of groups were tried using k-means clustering algorithm on a MDS visualization of the scaled by Z-score of the 88 tumor samples for the hallmark inflammatory response signature from MSigDB, containing 200 genes. ICAM4 was notably not detected in the dataset in the TARGET-OS cohort. Thus, 199 genes from the signature were used.

The groups are chosen from Figure 1.C as they are relevant as they correspond fairly well to functional groups, defined by k-means clustering based on MDS visualization **(Figure 1)**. Each sample is thus attributed to its inflammatory status and this group will be subsequently used for the following results.

1.2 Characterization of osteosarcomas associated to inflammatory status

In order to characterize osteosarcomas, the *Hp Osteosarcoma* gene signature from MSigDB was used to see whether the inflammatory groups can be related to gene expression from this signature. Visually, the heatmap representing the gene signature, annotated with the inflammatory groups, does seem to indicate that the samples express different genes between Low, Medium and High inflammation group (**Fig. 2A**). However, the dendrogram clustering the samples indicates that the gene signature does not cluster well with the inflammatory annotations. However, despite high heterogeneity between samples and inflammatory status,

However *Hp Osteosarcoma* in GSEA is not significantly differrent ($p = 0.33$).

Comparison of the expression of specific osteosarcoma markers relating to osteoblastic, chondroblastic, fibroblastic markers has also been done, through a heatmap representation. Hierarchical clustering of the samples does not appear to be associated with corresponding inflammatory status. However it does reveal that there are groups of osteoblastic, chondroblastic and fibroblastic osteosarcomas which is expected (**Fig. 2C**).

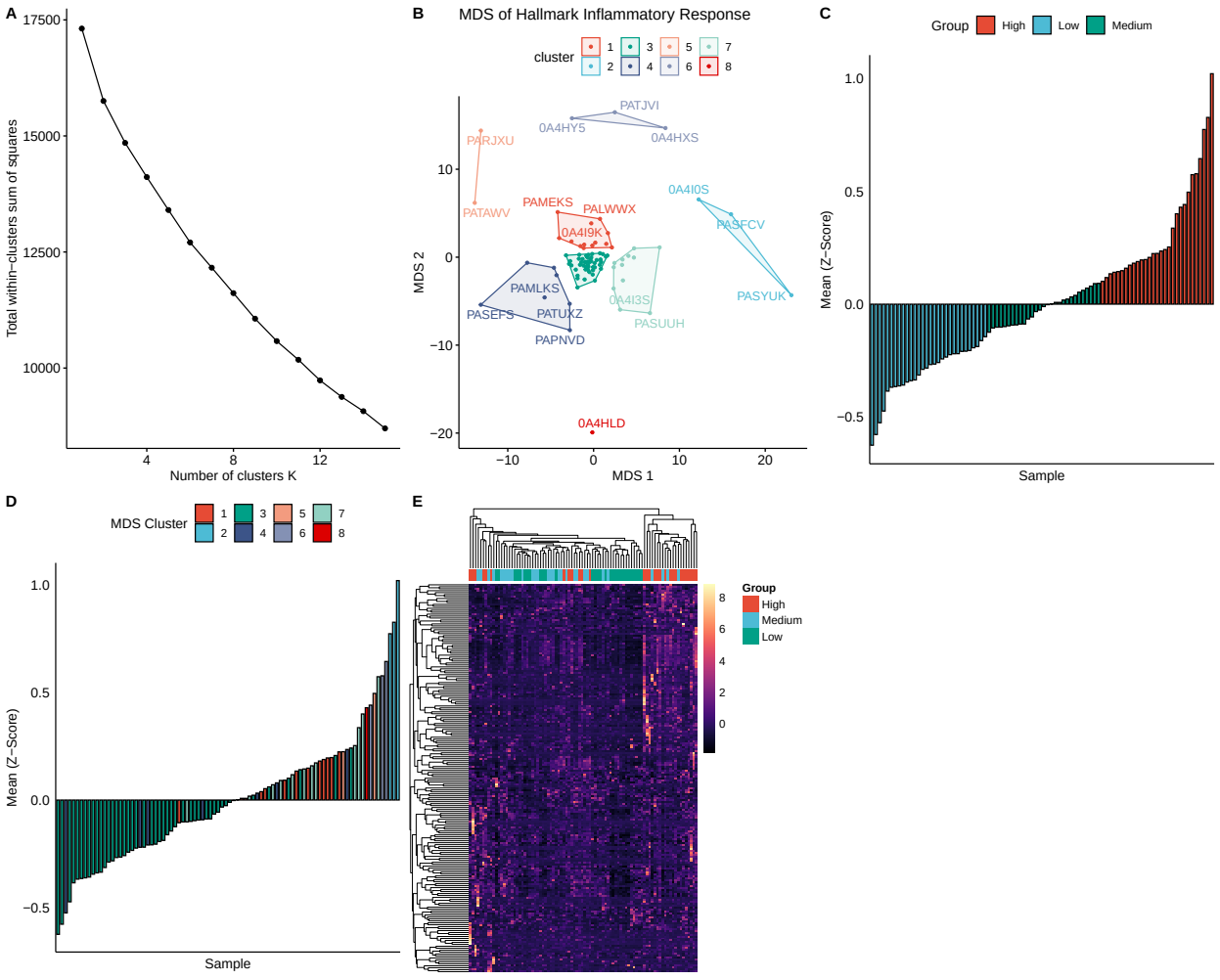

Figure 1: Construction of inflammatory groups

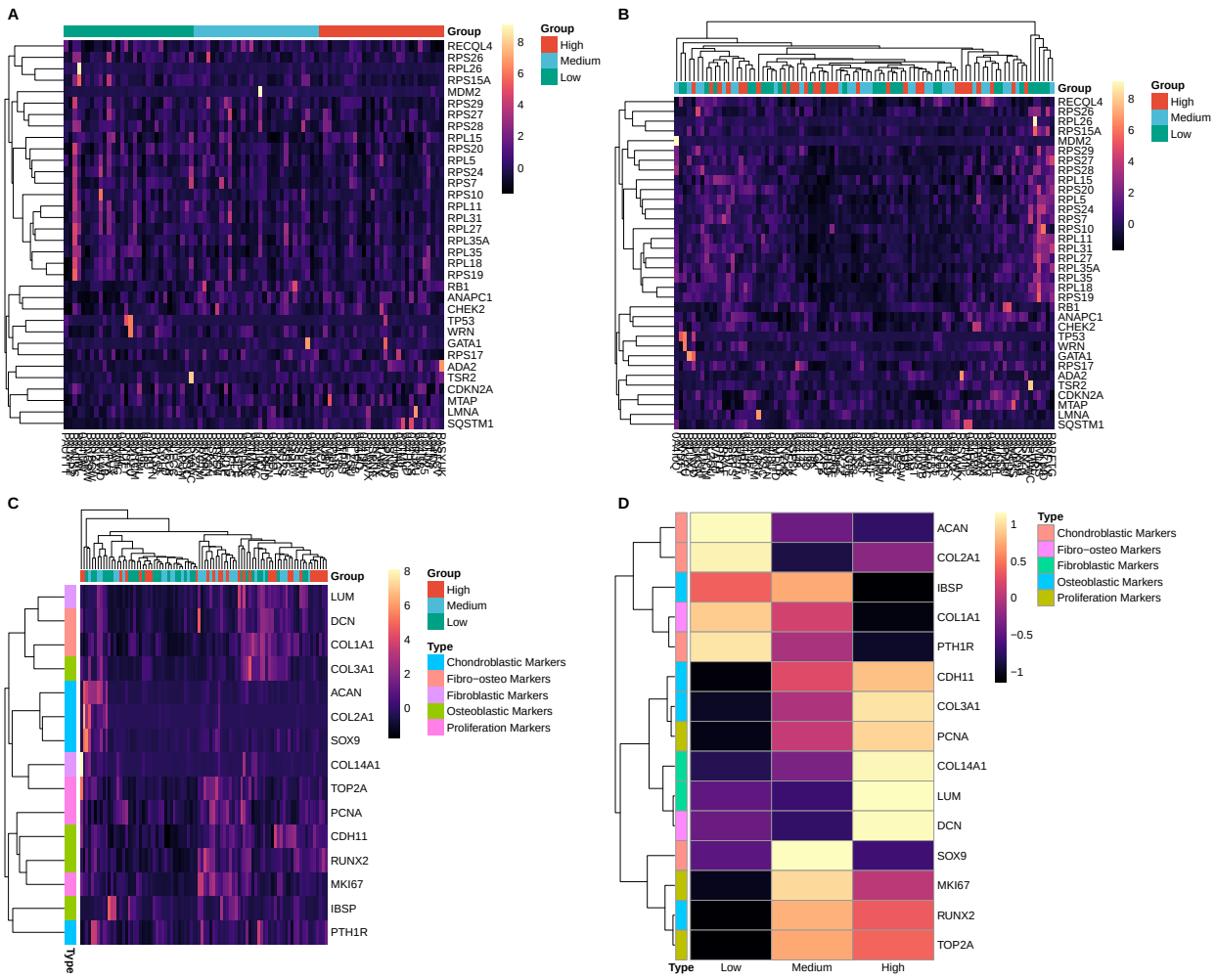

Figure 2: **Heatmap of HP Osteosarcoma signature with samples annotated to their respective inflammatory groups.**

The mean of markers of proliferation (MKI67, PCNA, TOP2A) associated to osteosarcomas have been compared to inflammatory status, along with the mean of the three markers. Kruskal-Wallis testing is significant (p = 0.00968) and post-hoc Dunn analysis reveals that the mean of the proliferation markers between low and high group is significantly different (p = 0.016). The data suggests that proliferation is hindered when inflammatory status is high in the osteosarcoma samples.

1.3 Characterization of intra-tumor inflammation associated to inflammatory status

1.3.1 General relationship of inflammatory status with immune response

1.3.1.1 Relationship with ESTIMATE and inflammatory signatures Using ESTIMATE algorithm from *tidyestimate* R package, an immune score has been calculated for each sample which reflects the immune infiltration in a given tumor sample. The violin plot represents the values obtained for each inflammatory group **(Fig. 3)**.

```
vep <- ggarrange(ve, labels = "AUTO")
suppressWarnings(print(vep))
```

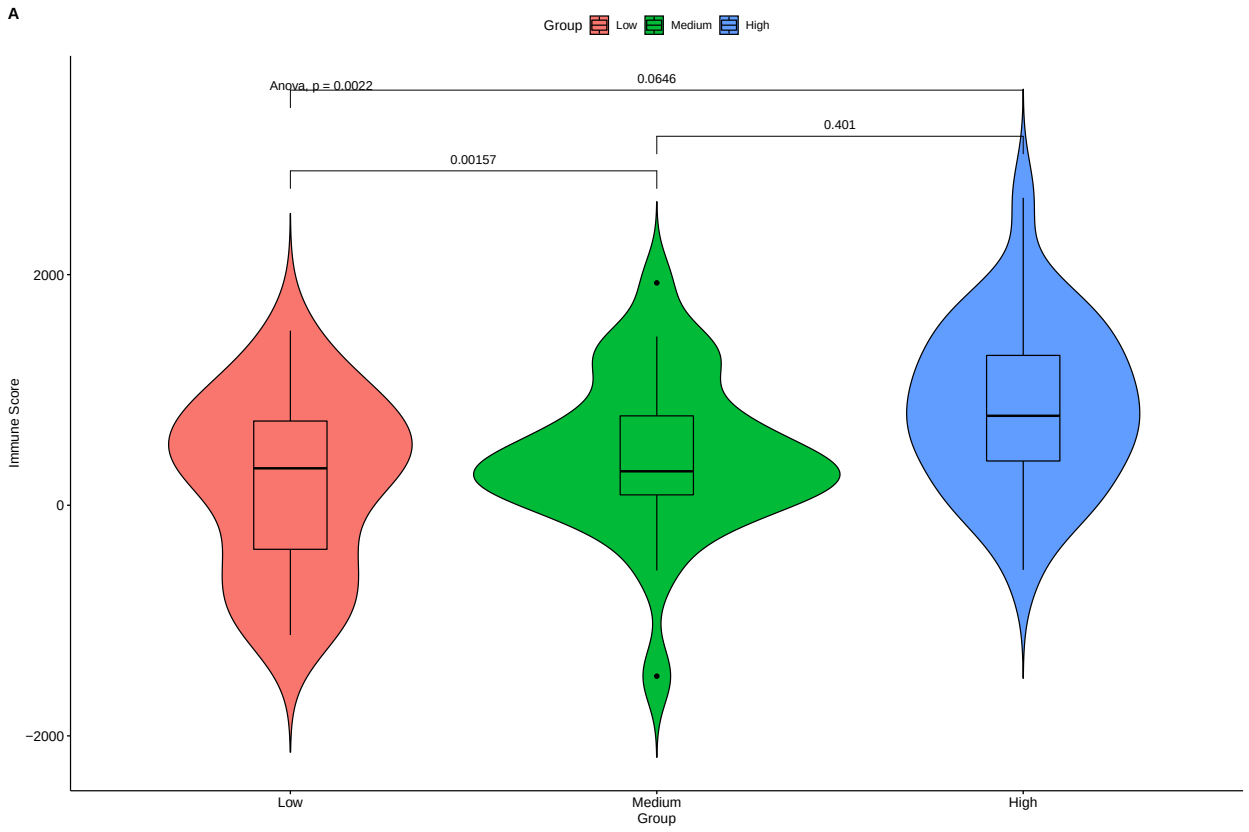

Figure 3: **Violin plot of ESTIMATE score for each inflammatory group.** ANOVA was performed followed by Tukey's post-hoc analysis. P < 0.05 is considered statistically significant.

1.3.1.2 Immune abundance by immune deconvolution algorithm Immune cell abundance can be determined thanks to immune deconvolution algorithm. Here, MCP-Counter CIBERSORTx and xCell have been tried.

```
immune_plot <- ggarrange(mcp_plot, ciber_plot, ciber_tidy, labels = "AUTO")
suppressWarnings(print(immune_plot))
```

```
suppressWarnings(print(ggsurv))
```

1.3.1.3 Survival curve

1.3.2 Similarity of inflammatory signatures

```
knitr::include_graphics("../04_figures/ggplot/Venn_estimate_vs_hallmark_inflammatory_response.png")
```

```
knitr::write_bib(c(.packages()), "../R-Markdown-Report/bibliography/packages.bib")
```

```
## Warning in meta$Date: correspondance partielle de 'Date' en 'Date/Publication'

## Warning in meta$Date: correspondance partielle de 'Date' en 'Date/Publication'
```

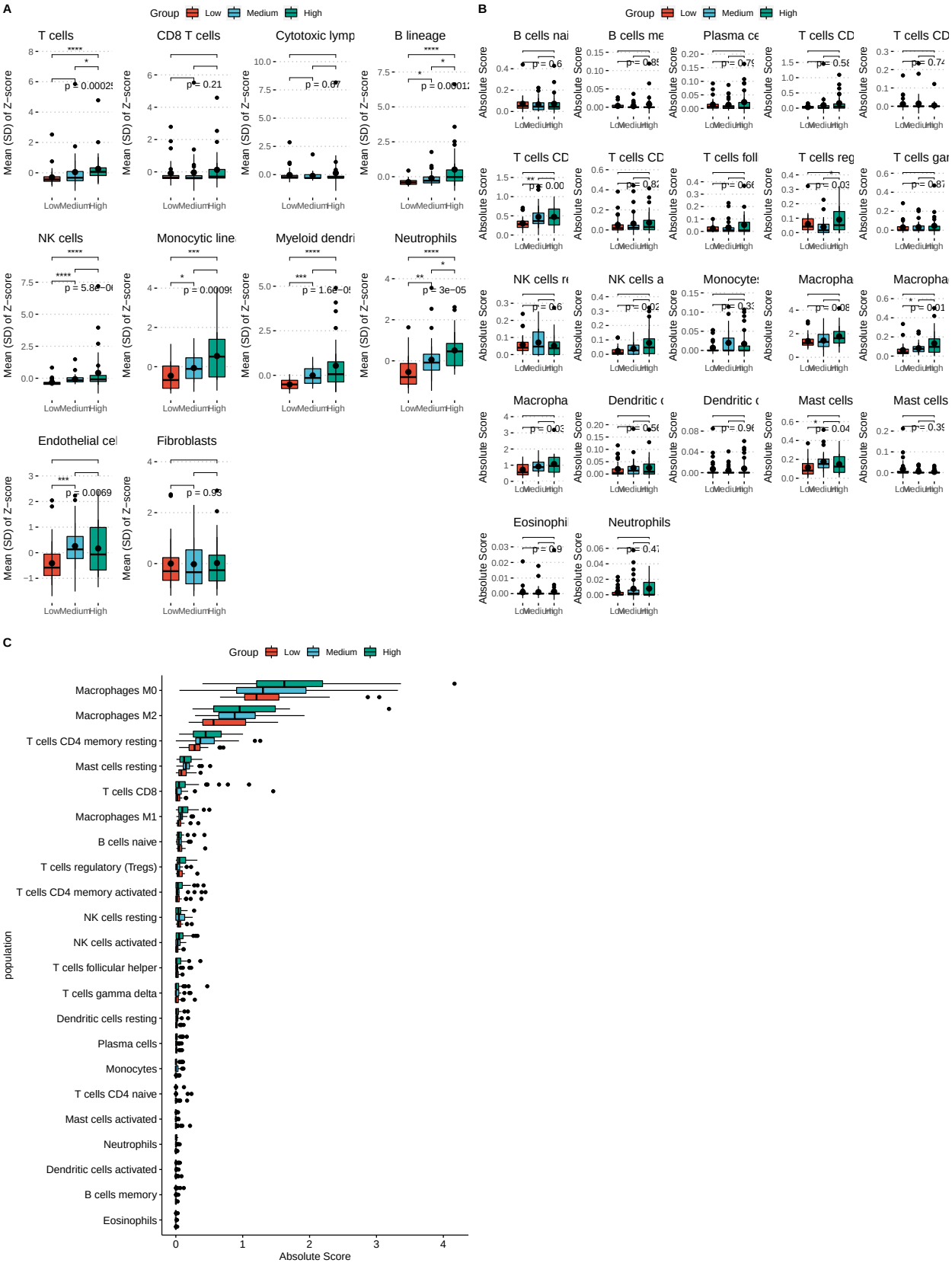

Figure 4: Immune deconvolution plot. (A) MCP-counter

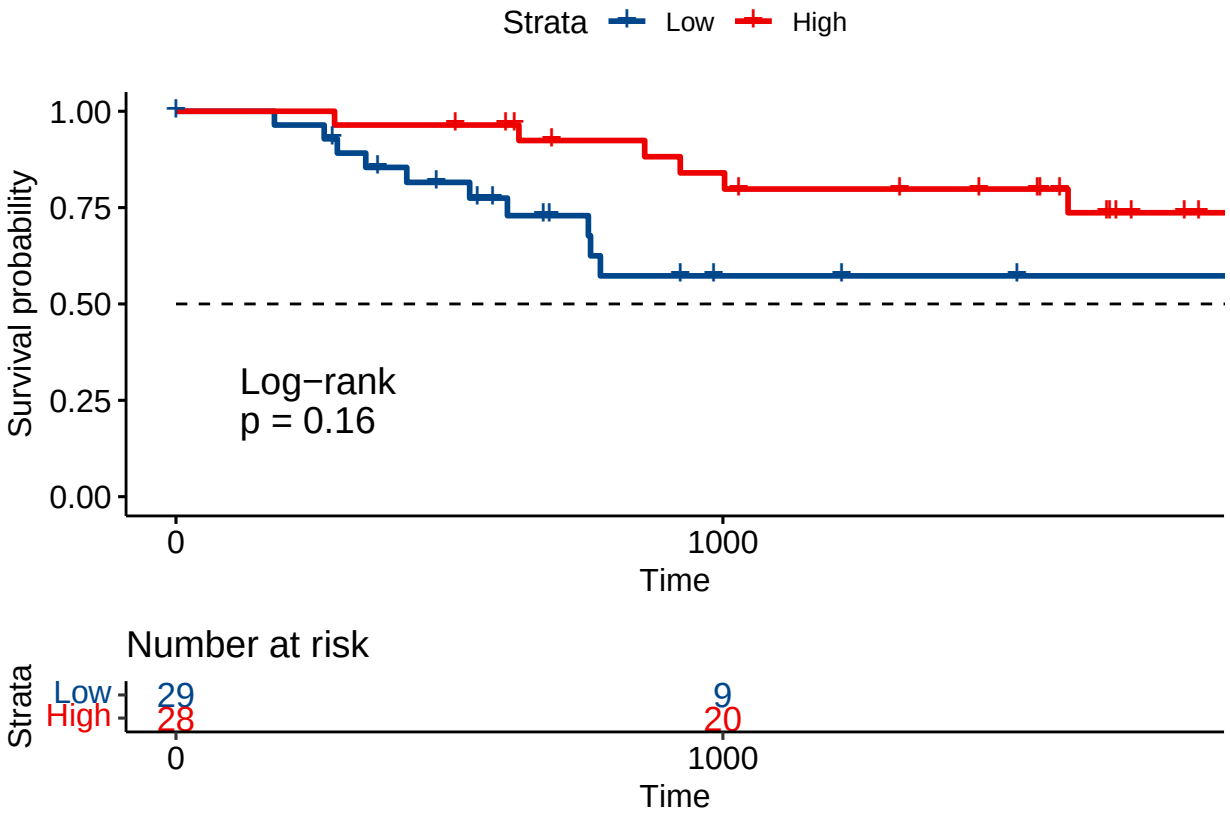

Figure 5: **Kaplan-Meier survival plot of low vs high group indicate a trend associating inflammatory status with survival prognostic.** Horizontal and vertical axes represent survival times and rates, respectively. Red and blue curves are samples with risk score higher and lower than the median value, respectively. Plus signs indicate censored values. Depicted P-values were obtained by the logrank test.

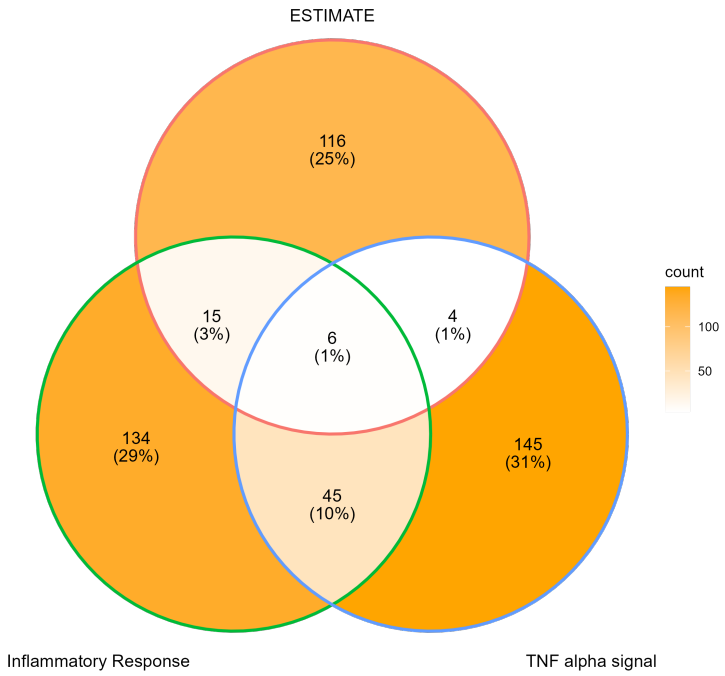

Figure 6: Venn diagram comparing three inflammatory signatures.