Neural Progenitor Cells for Treatment of Spinal Cord Injury

Executive summary

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Data modifications

- **Normalisation:** Expression value for each day, animal and target was divided by the mean of the expression for the same target in healthy (none injured, none transplanted) animals.
- **Outliers:** All observations with normalised expression > 20 were removed. This cut-off was based on appearance of histogram. IL-7 was completely removed due to heavy influence of outliers.

Statistical analysis

Evaluation of assumptions

- **Assumption of normality** was evaluated using *Shapiro Wilk's* test for each treatment (group) within each time point and for each target separately. Null hypothesis that data is normally distributed was rejected at the 5 % level.
- **Assumption of homogenity** of variances was evaluated between the two treatments within each time point and for each target was evaluated using *Fligner Killeen's* test. Null hypothesis that the variances were equal was rejected at the 5 % level.

Independent intraday two group comparison

- **Two-sided non-paired Student's t-test** was used for group comparison given that data in both treatments was normally distributed and the variances were equal between the treatments. Given that data for both treatments was normally distributed but the groups had unequal variances the variance was estimated separately for each group and the Welch modification to the degrees of freedom was used.
- **Two-sided non-paired Wilcoxon Rank Sum test** was used with continuity correction in the normal approximation for the p-value was used given that data in at least one of the treatments was not normally distributed.

Agglomerative hierarchical clustering

- Average expression for each cytokine/chemokine, week and treatment were clustered using agglomerative hierarchical clustering and presented with heatmap.

Independent multiple group within treatment comparison over time

- **One-way ANOVA** was used in case the data was normally distributed at all time points for a target and treatment, and the variances where homogenous between the treatments. In case the data was normally distributed but the variances were not homogenous the difference was assessed using Welch ANOVA. Assumptions of normality and homogenity of variances were evaluated as described above. One-way ANOVA was assumed to be robust against violations of the normality assumption.

- Tukey's post hoc test was used for post hoc comparisons following one-way ANOVA. Games-Howell test was used for post hoc comparisons following Welch ANOVA.

Open source access

R-script and html-report can be accessed at github. Please feel free to fork or make a pull request.

Pro-inflammation over time

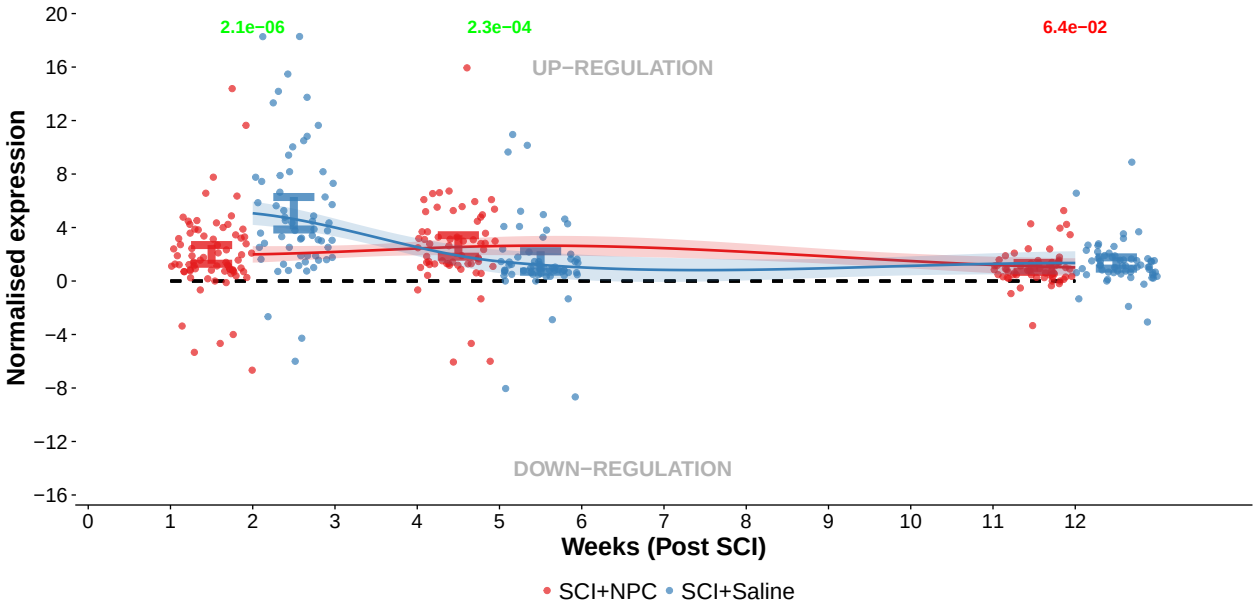


Figure 1: Figure expression of pro-inflammatory cytokines/chemokines (IL-1a, IL-1b, IL-5, IL-6, IL-12(p70), IL-17, IL-18, GM-CSF, GRO/KC, IFN-g, MCP-1, MIP-1a, MIP-3a, RANTES, TNF-a) over time for each treatment group. Raw expression was normalised to average expression in healthy control for each target, animal and time point separately. Normalised values > 20 and < -10 were considered outliers and removed. P-values for independent two group comparison is presented at each time point. P-values are median p-values of 1000 two-group comparisons of 1000 bootstrapped data samples for each treatment. Assumptions and test selection as described above.

Sensitivity analysis of comparison between treatments on pro-inflammation over time

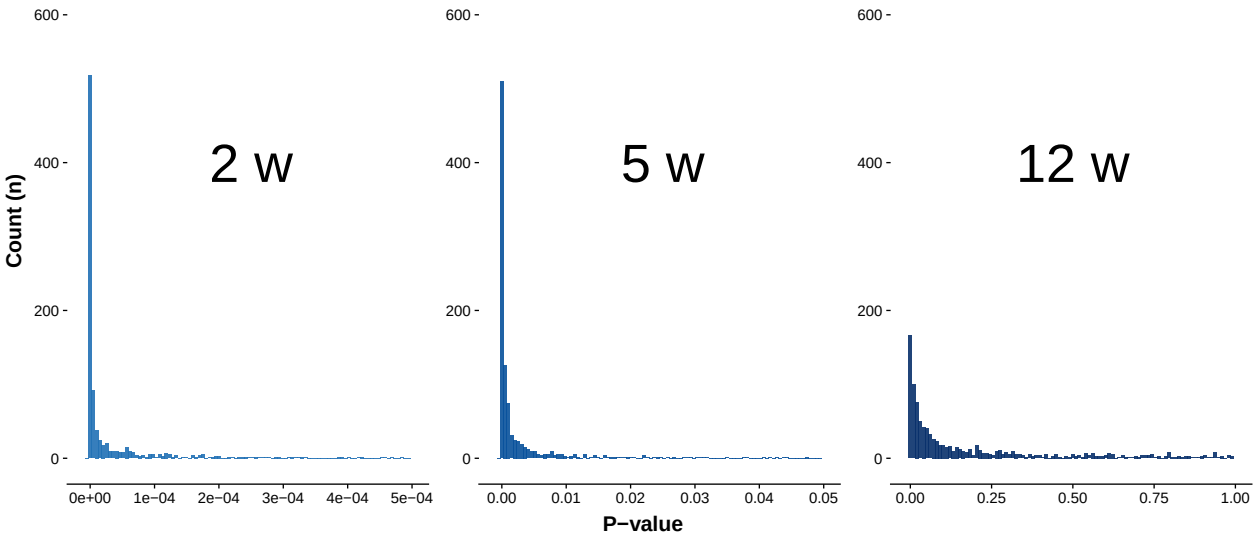


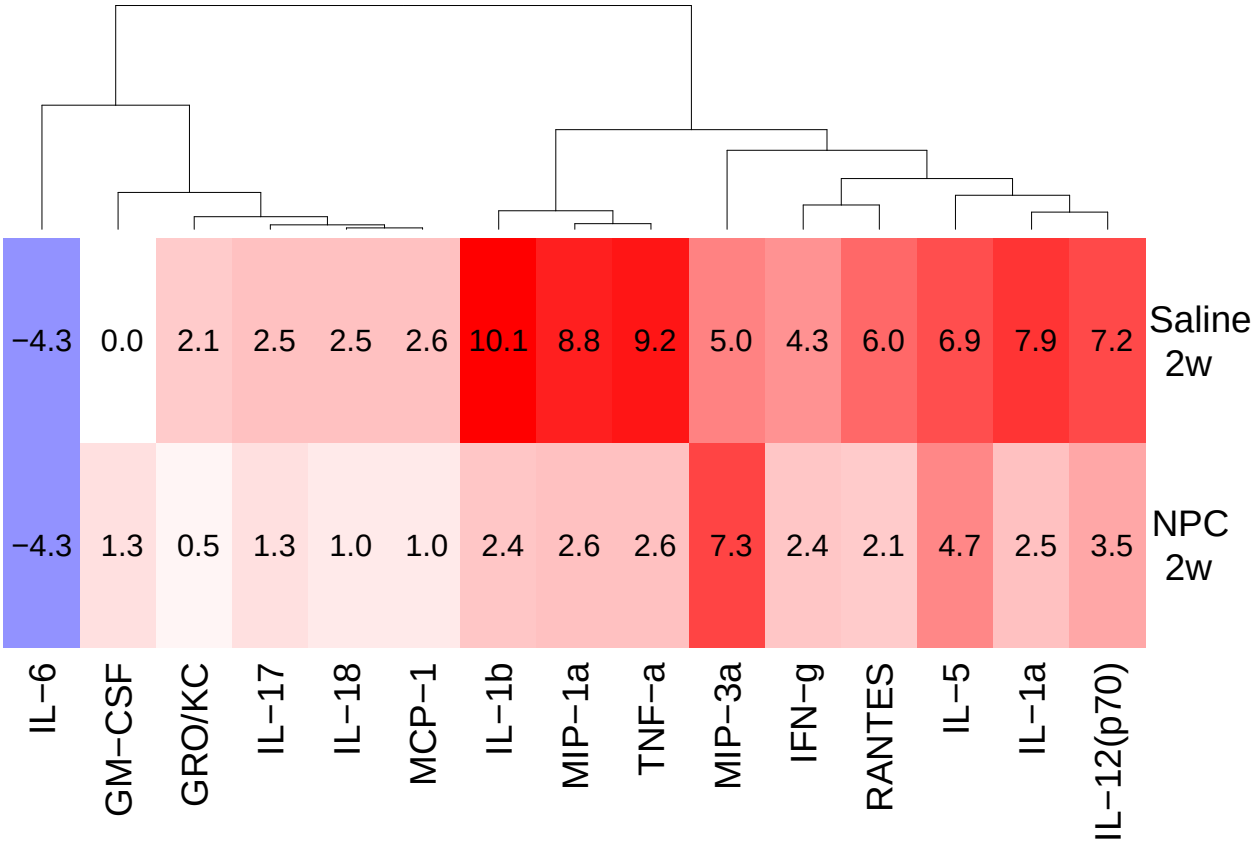
Figure 2: Figure reports histograms (bins=100) of 1000 p-values for two group comparison calculated on bootstrapped data for pro-inflammation from each treatment at each time point.

2w	5w	12w
2.1e-06	2.3e-04	6.4e-02

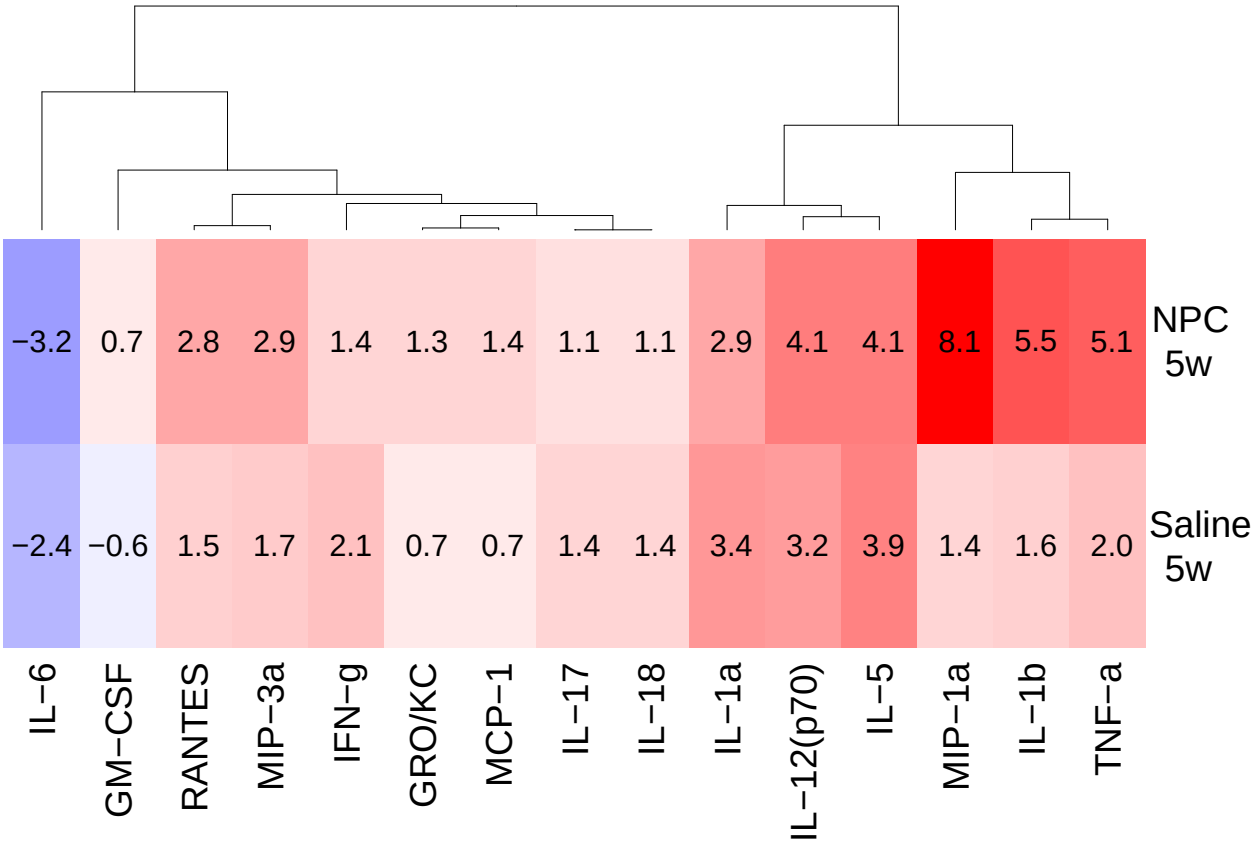
Table 1: Median p-values of 1000 p-values for two group comparison calculted on bootstrapped data for pro-inflammation from each treatment at each time point.

Agglomerative hierarchical clustering

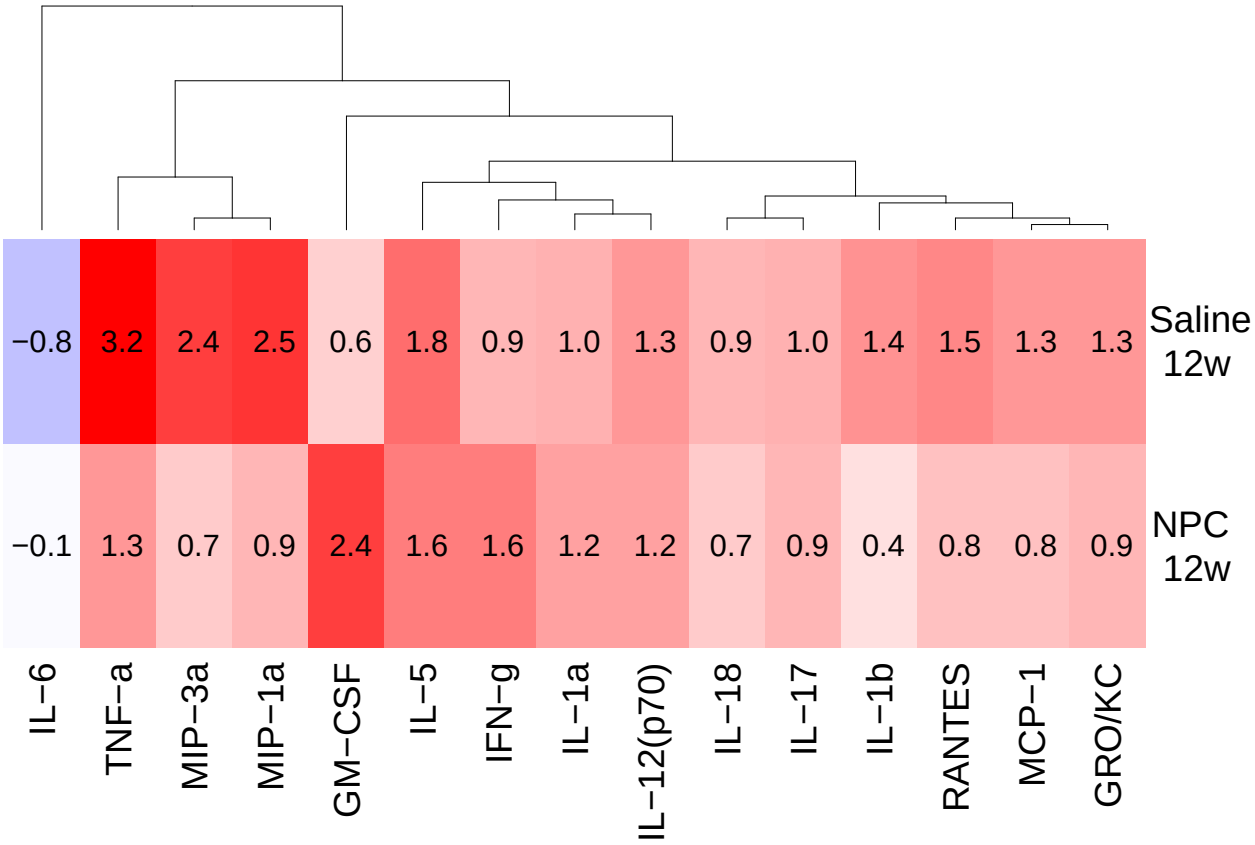
2 weeks post SCI



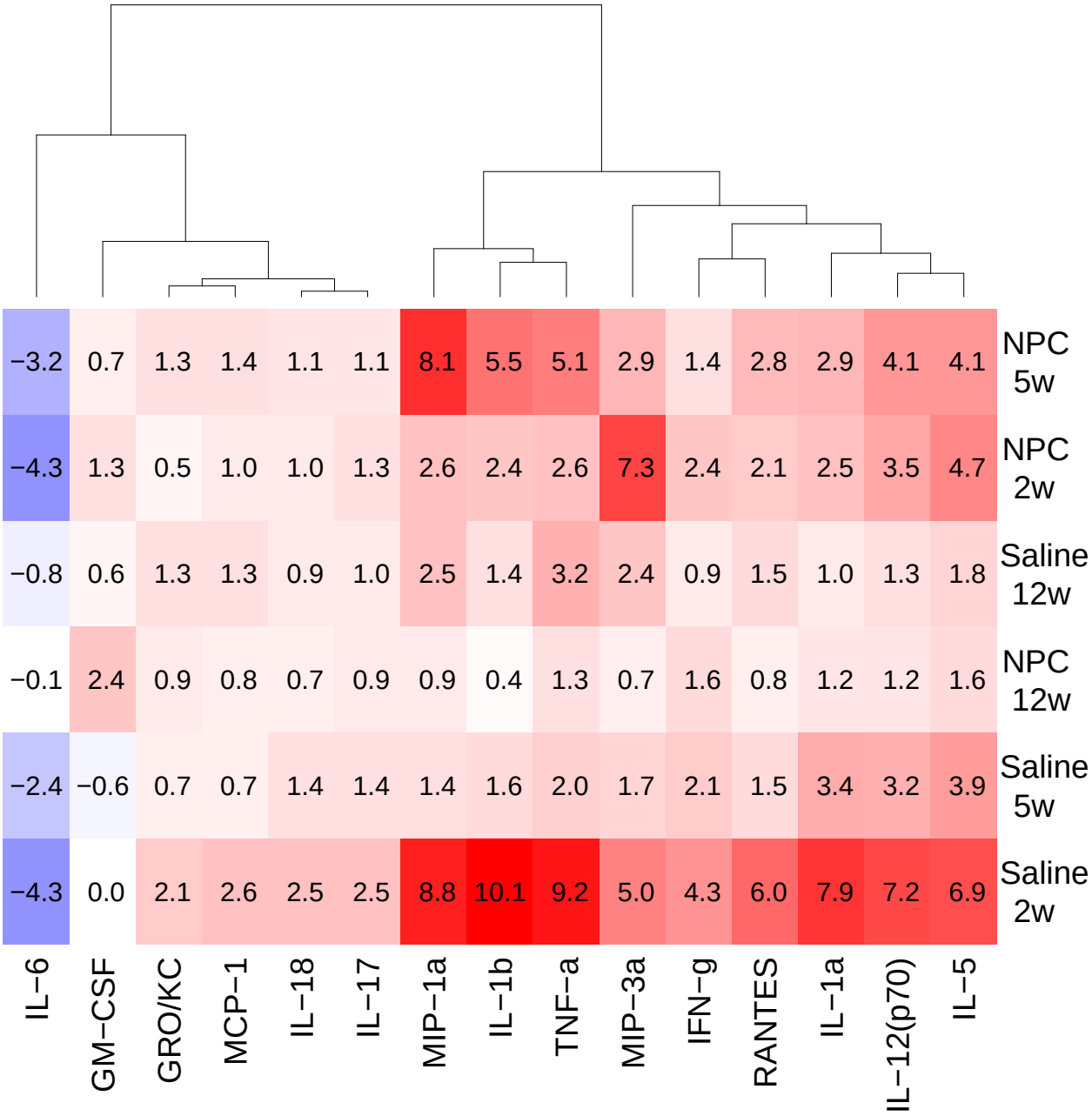
5 weeks post SCI



12 weeks post SCI

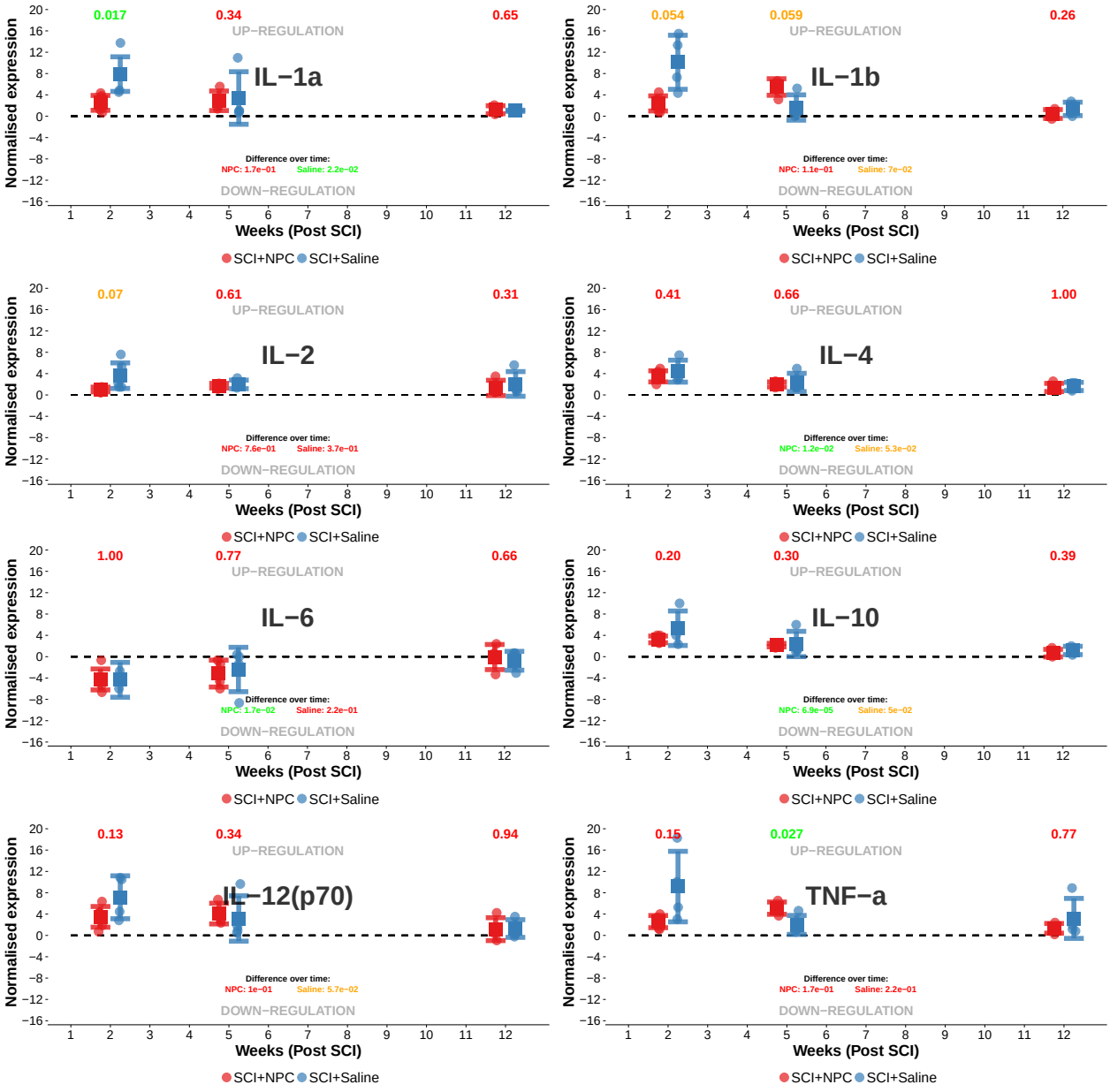


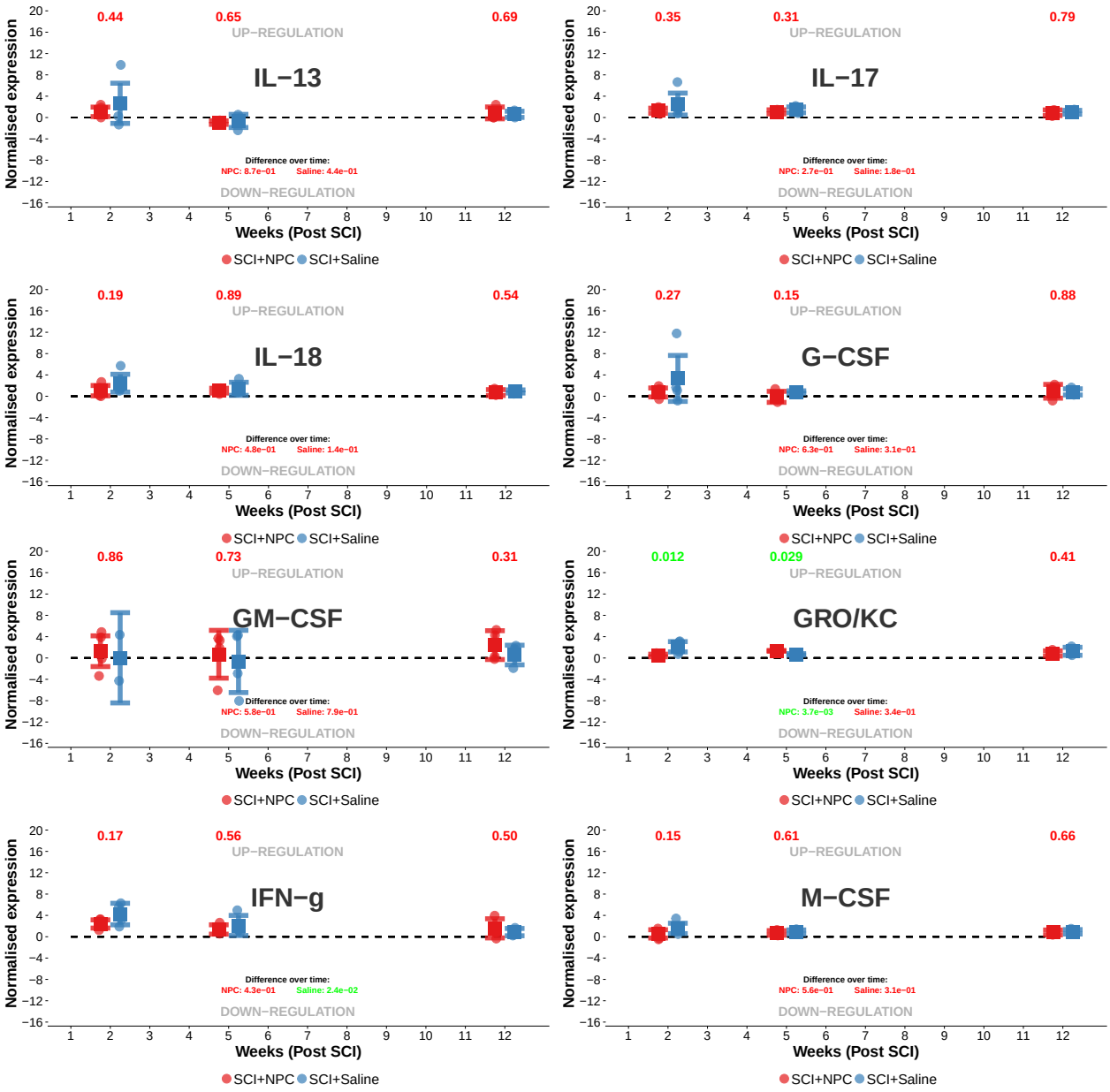
2, 5 and 12 weeks



Figur 3: Figure reports agglomerative hierarchical clustering with heatmap of pro-inflammatory cytokines/chemokines for each treatment and time point. Values are average normalised expression (raw expression divided by the average expression in healthy for each target, animal and week separately).

Individual cytokines over time




0.44
0.65
0.69
UP−REGULATION
IL−13
Difference over time:
NPC: 8.7e−01
Saline: 4.4e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.35
0.31
0.79
UP−REGULATION
IL−17
Difference over time:
NPC: 2.7e−01
Saline: 1.8e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.19
0.89
0.54
UP−REGULATION
IL−18
Difference over time:
NPC: 4.8e−01
Saline: 1.4e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.27
0.15
0.88
UP−REGULATION
G−CSF
Difference over time:
NPC: 6.3e−01
Saline: 3.1e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.86
0.73
0.31
UP−REGULATION
GM−CSF
Difference over time:
NPC: 5.8e−01
Saline: 7.9e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.012
0.029
0.41
UP−REGULATION
GRO/KC
Difference over time:
NPC: 3.7e−03
Saline: 3.4e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.17
0.56
0.50
UP−REGULATION
IFN−g
Difference over time:
NPC: 4.3e−01
Saline: 2.4e−02
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline
0.15
0.61
0.66
UP−REGULATION
M−CSF
Difference over time:
NPC: 5.6e−01
Saline: 3.1e−01
DOWN−REGULATION
Normalised expression
Weeks (Post SCI)
SCI+NPC
SCI+Saline

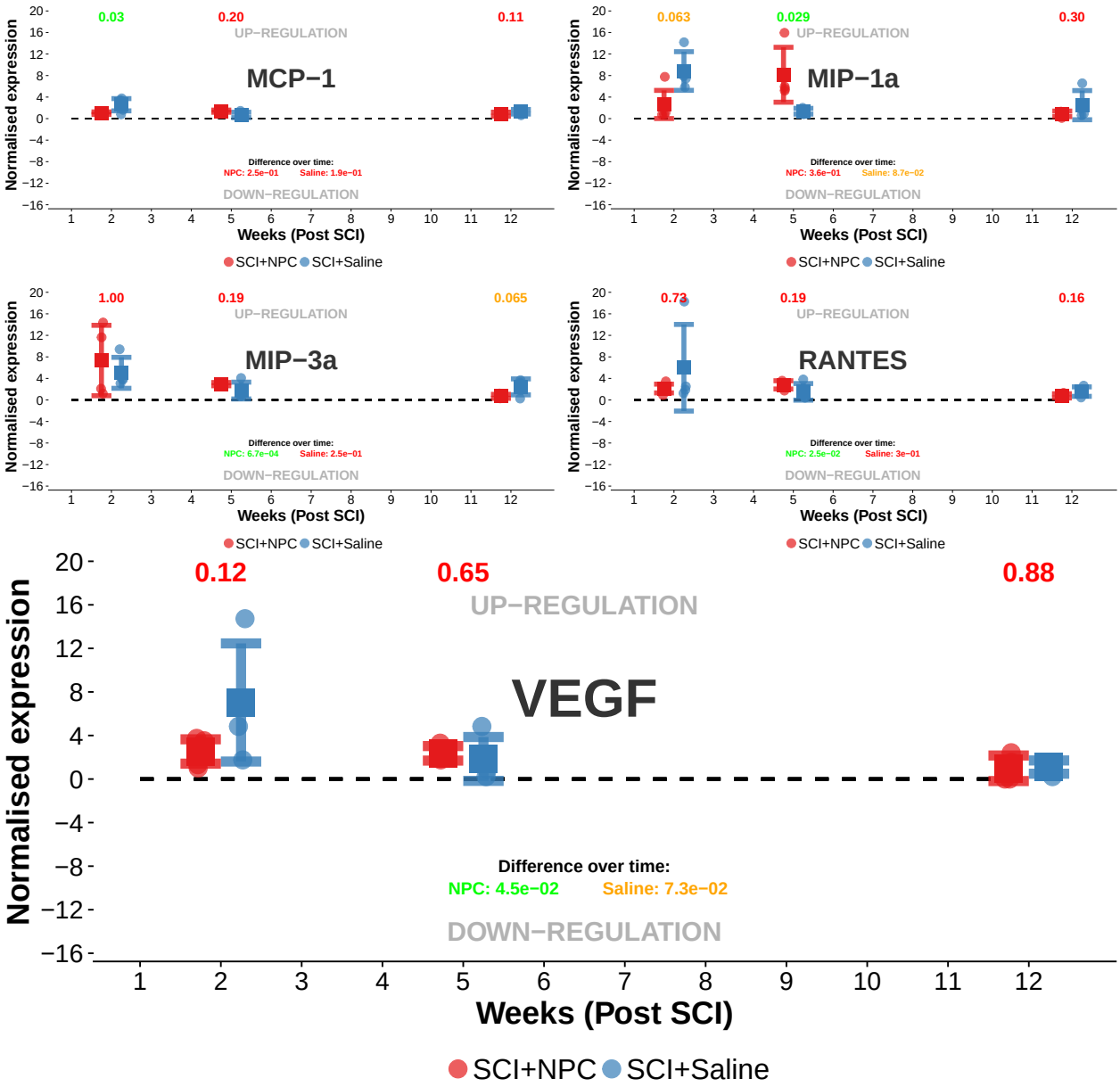


Figure 4: Each plot reports expression of one cytokine per treatment group and time point. Statistical analysis as described above. P-values for comparison of the two independent groups are presented at each time point. Color of p-value is gree if p-value < 0.05, orange if p-value>0.05 & p-value <0.1 and red if p-value >0.1. P-values for within treatment multiple comparison (over time) are presented in the lower part of the plot.

```
sessionInfo()
```

```
## R version 3.4.1 (2017-06-30)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.3 LTS
##
## Matrix products: default
## BLAS: /usr/lib/libblas/libblas.so.3.6.0
## LAPACK: /usr/lib/lapack/liblapack.so.3.6.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=sv_SE.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=sv_SE.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=sv_SE.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=sv_SE.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] gplots_3.0.1        gridExtra_2.3       knitr_1.17
## [4] cowplot_0.9.1       RColorBrewer_1.1-2  data.table_1.10.4-3
## [7] ggplot2_2.2.1
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.13       magrittr_1.5       munsell_0.4.3
##  [4] colorspace_1.3-2   rlang_0.1.2        highr_0.6
##  [7] stringr_1.2.0      plyr_1.8.4         caTools_1.17.1
## [10] tools_3.4.1        gtable_0.2.0       KernSmooth_2.23-15
## [13] gtools_3.5.0       htmltools_0.3.6    yaml_2.1.14
## [16] lazyeval_0.2.0     rprojroot_1.2      digest_0.6.12
## [19] tibble_1.3.4       bitops_1.0-6       evaluate_0.10.1
## [22] rmarkdown_1.6      labeling_0.3       gdata_2.18.0
## [25] stringi_1.1.5      compiler_3.4.1     scales_0.5.0
## [28] backports_1.1.1
```