Neural Progenitor Cells for Treatment of Spinal Cord Injury

Executive summary

- Transplanted GFP+NPCs suppress the level of pro-inflammation in the spinal cord 2 weeks post SCI *more* than saline control. There is no difference in terms of effect on pro-inflammation at 5 and 12 weeks when comparing GFP+NPCs and saline control.
- The suppression in pro-inflammation observed 2 weeks post SCI and caused by NPCs was mainly driven by a suppression of IL-1a (p=0.013), IL-1b (0.0064), IL-2 (p=0.027), IL-12(p70) (p=0.082), TNF-a (p=0.016), GRO/KC (p=0.0049), MCP-1 (p=0.036), MIP-1a (p=0.0077) and IL-7 (p=0.05).

Data modifications

- **Log2 fold change:** Fold change in relation to healthy control was calculated for each each animal and target separately. Example for target X: I) mean expression for target X in healthy animals was calculated. II) The expression in animal Y for target X was divided by the mean expression of target X in healthy control (fold change). III) log2() was taken of the fold change.

Statistical analysis

Evaluation of assumptions

- **Assumption of normality** was evaluated for each target, treatment and time point separately. Example for target X at time point Y in time point Z: this is equivalent to one expression value per biological replicate. These values (n=4 or 5) was used in Shapiro Wilk's test for normality. Null hypothesis that data is normally distributed was rejected at the 5 % level.
- **Assumption of homogenity of variances** was evaluated for each target and time point separately. Example for target X at time point Y: this is equivalent to one expression value per animal for a total of two treatment groups, i.e. n=8 or 10 observations. The homogenity was assessed between the treatments within time point Y. Null hypothesis that the variances were equal was rejected at the 5 % level.

Independent intraday two group comparison

- Given that data in both treatment groups within one time point for a target was normally distributed and the variances were *equal* **two-sided non-paired Student's t test** was used for group comparison. Given that both data was normally distributed in both treatment groups within one time point for a target but the variances were *not equal* **two-sided non-paired Student's t test** with Welch modification to the degrees of freedom was used.
- Given that data in at least one of the treatment groups within one time point was not normally distributed a **two-sided non-paired Wilcoxon Rank Sum test** was used to evaluate the difference.

Graphical presentation

- Mean in errorbars are mean of rat (biological replicates). Confidence intervals are 95 % and are based on the biological replicates only.

Agglomerative hierarchical clustering

- Average expression for each target, time point 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**. One-way ANOVA was assumed to be robust against violations of the normality assumption.

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

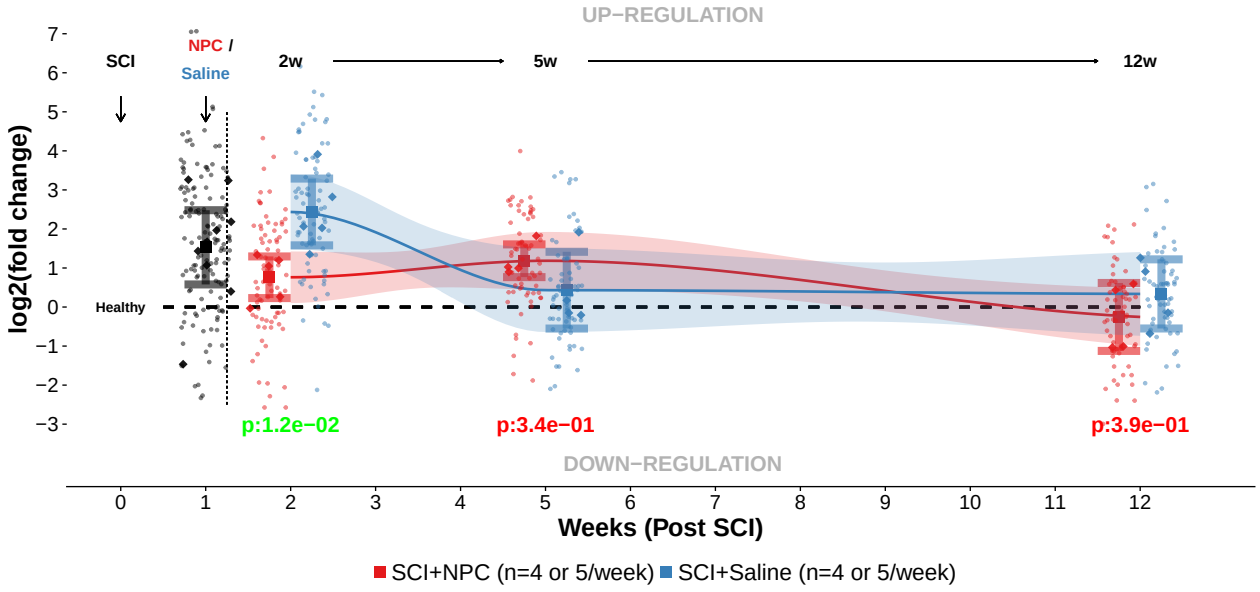


Figure 1. Figure log2(fold change in expression in relation to mean expresion in healthy control) of pro-inflammatory cytokines/chemokines (IL-1a, IL-1b, IL-5, IL-6, IL-12(p70), IL-17, IL-18, GM-CSF, GRO/KC, IFN-g, MCP-1, MIP-1a, MIP-3a, RANTES, TNF-a) over time for each treatment group. P-values for 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.

Distribution of mean based boostrapped data at each time point

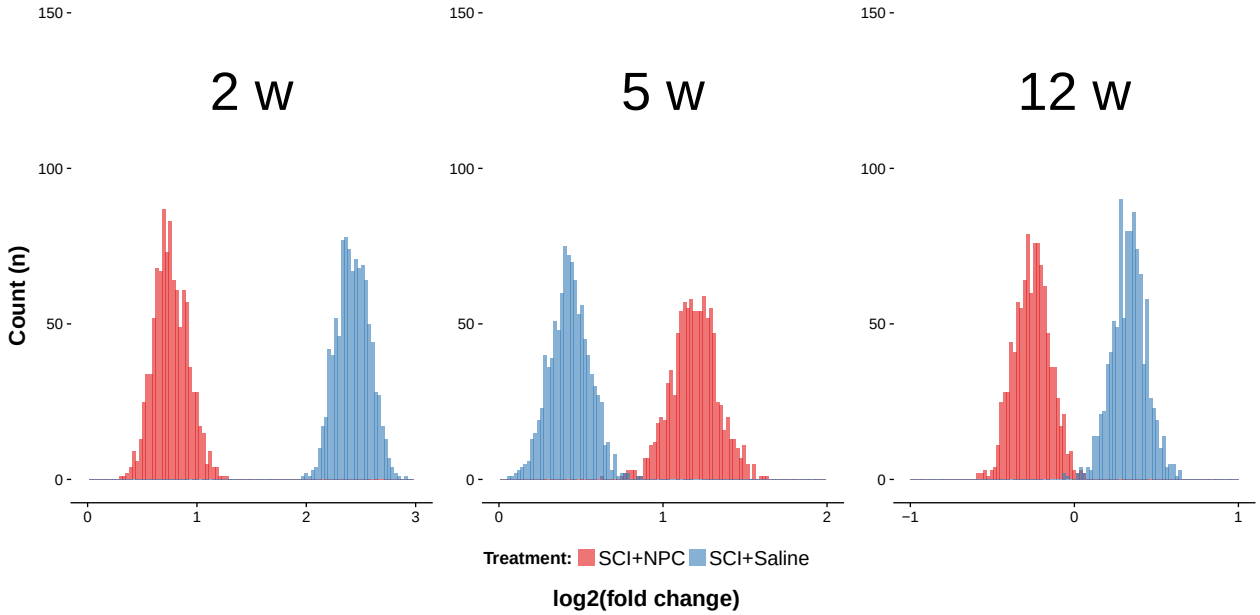
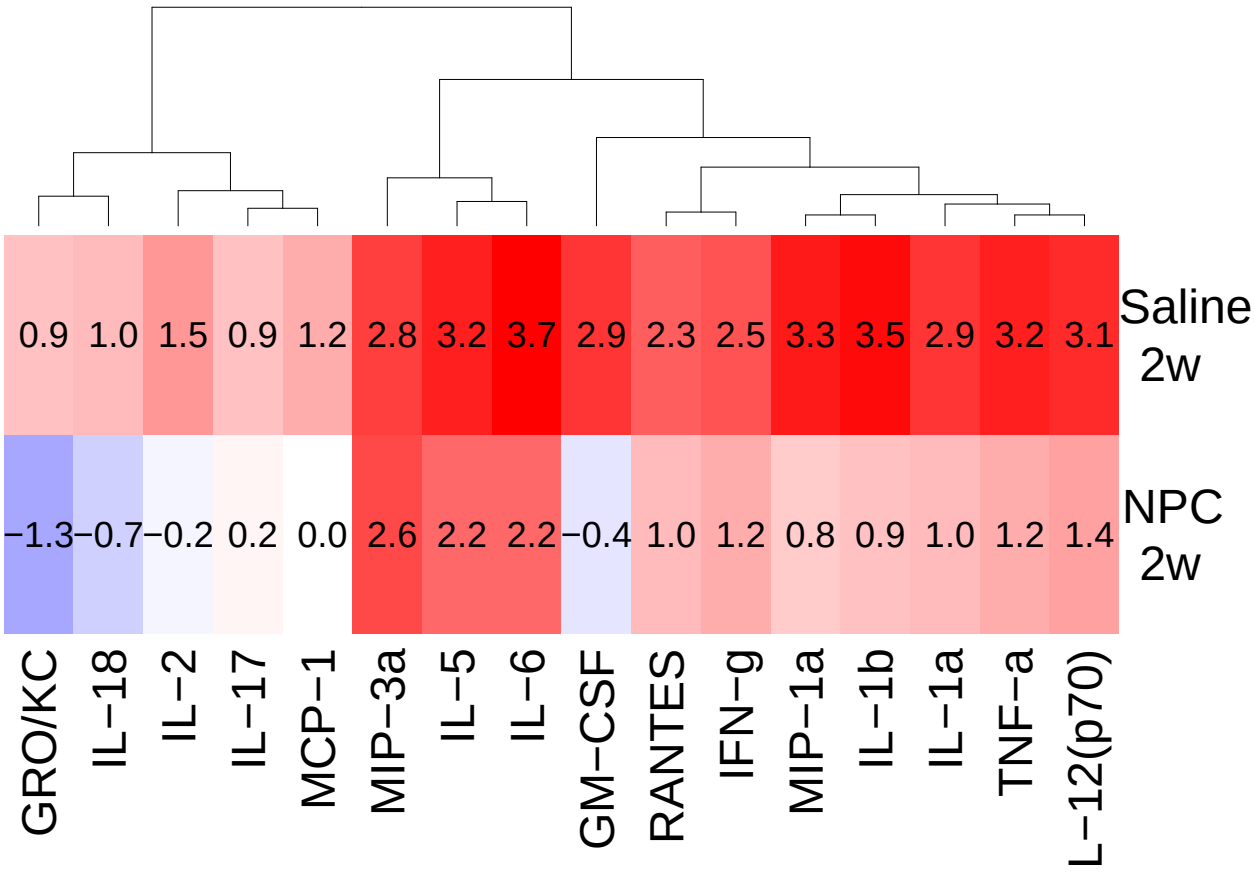


Figure 2. Figure reports histograms (100 bins) of 1000 mean log2(fold change) for each treatment and time point. One repeat in the analysis was created by I) bootstrapping data for each animal and time point (pro-inflammatory targets only), II) calculation of mean log2(fold change) per rat and time point, III) calculating the mean log2(fold change) per treatment and time point.

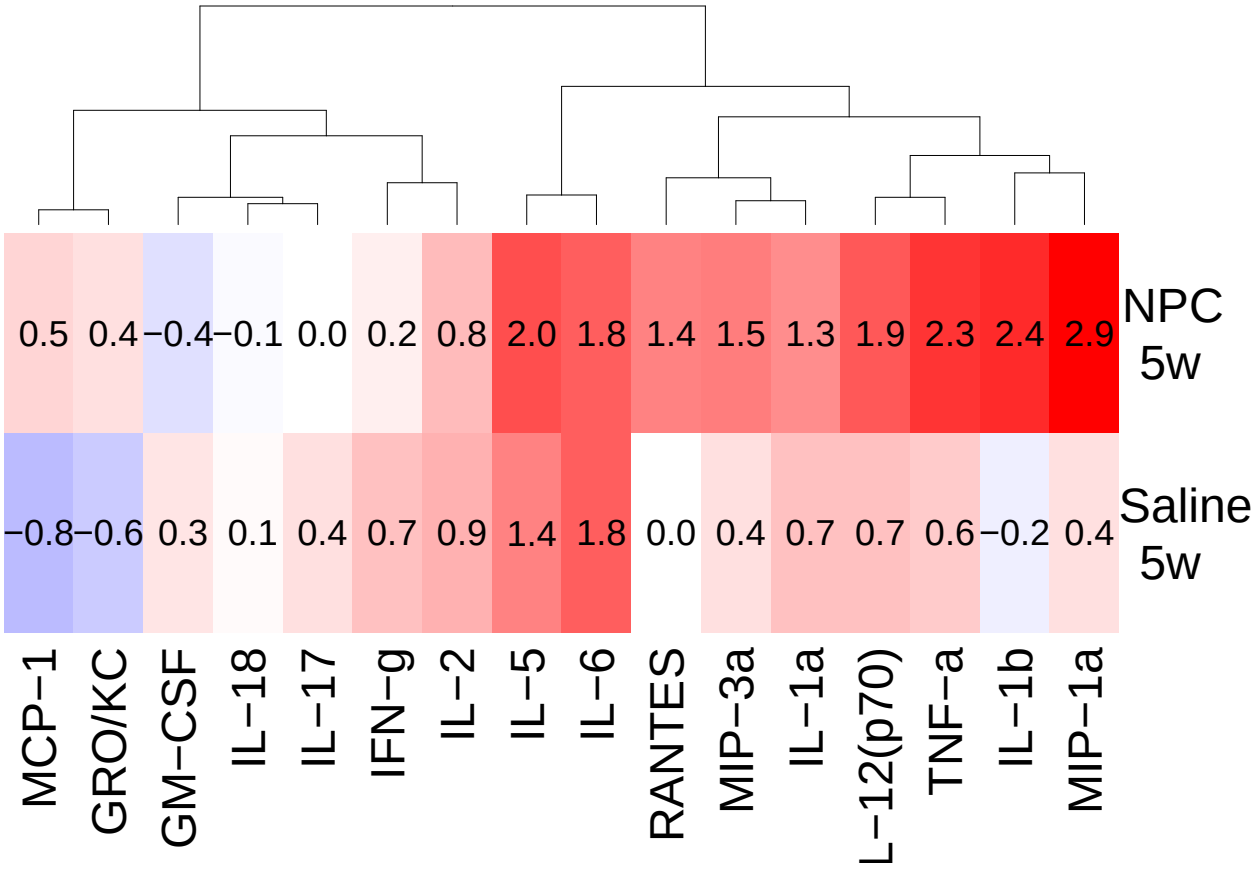
Treatment	2w	5w	12w
NPC	0.749	1.193	-0.254
saline	2.427	0.431	0.336

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

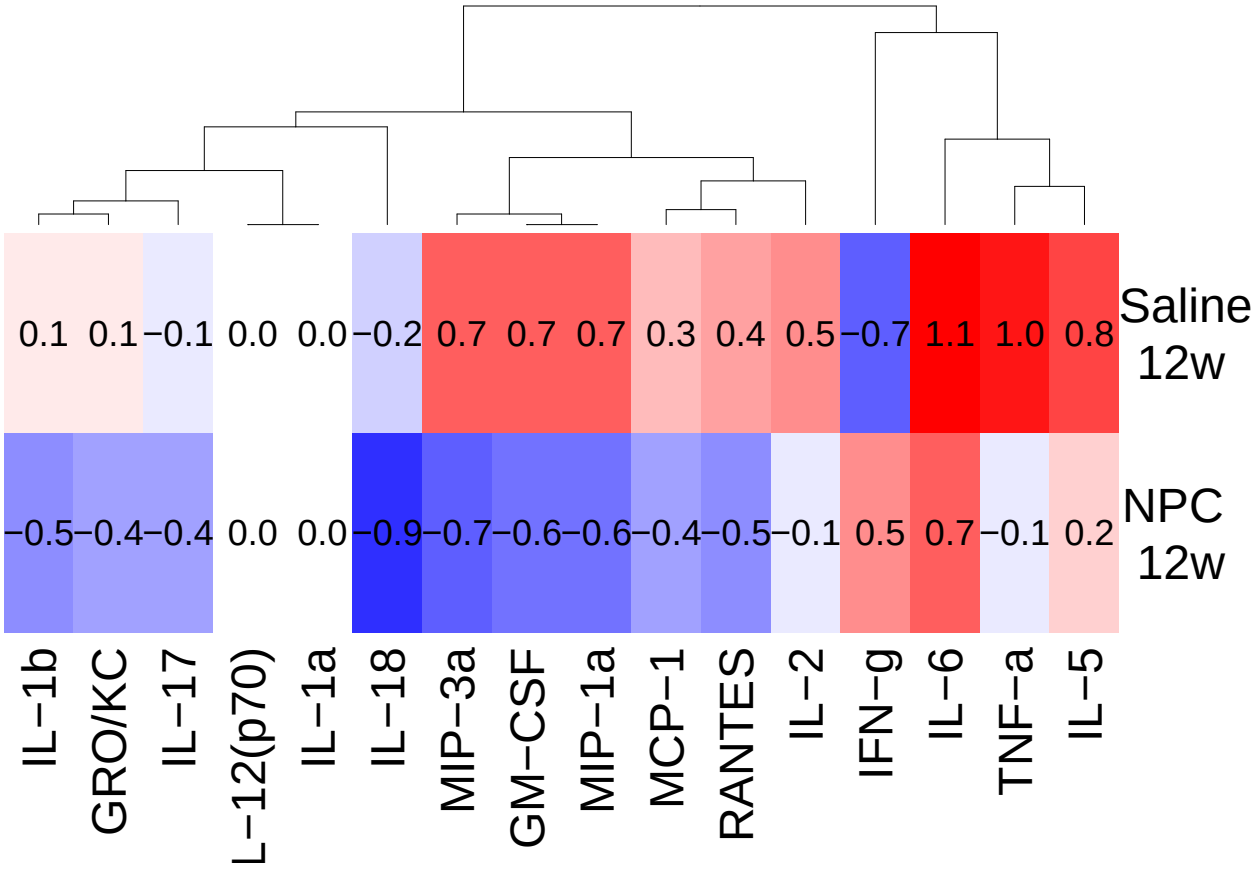
2 weeks post SCI



5 weeks post SCI



12 weeks post SCI



2, 5 and 12 weeks

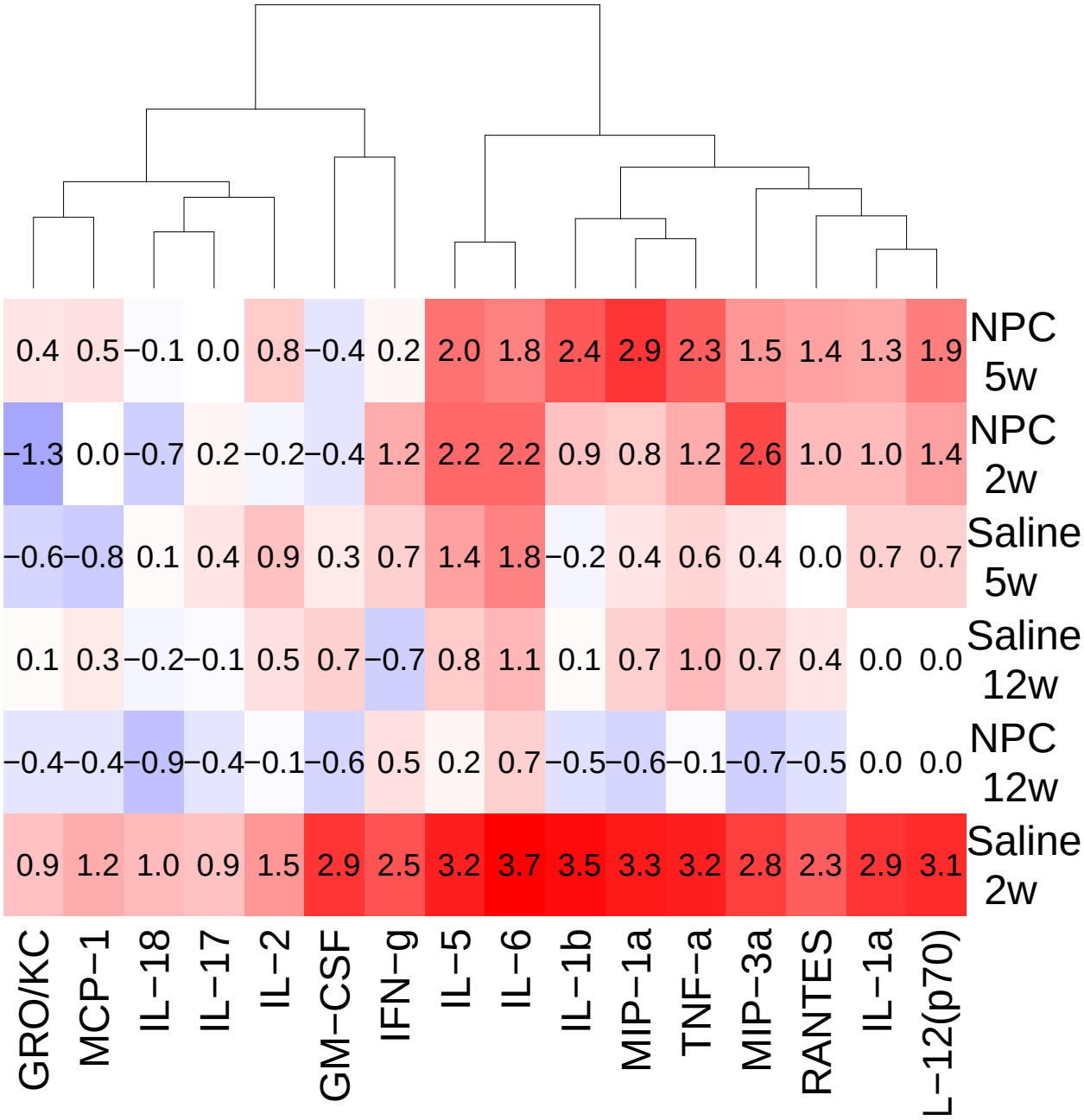
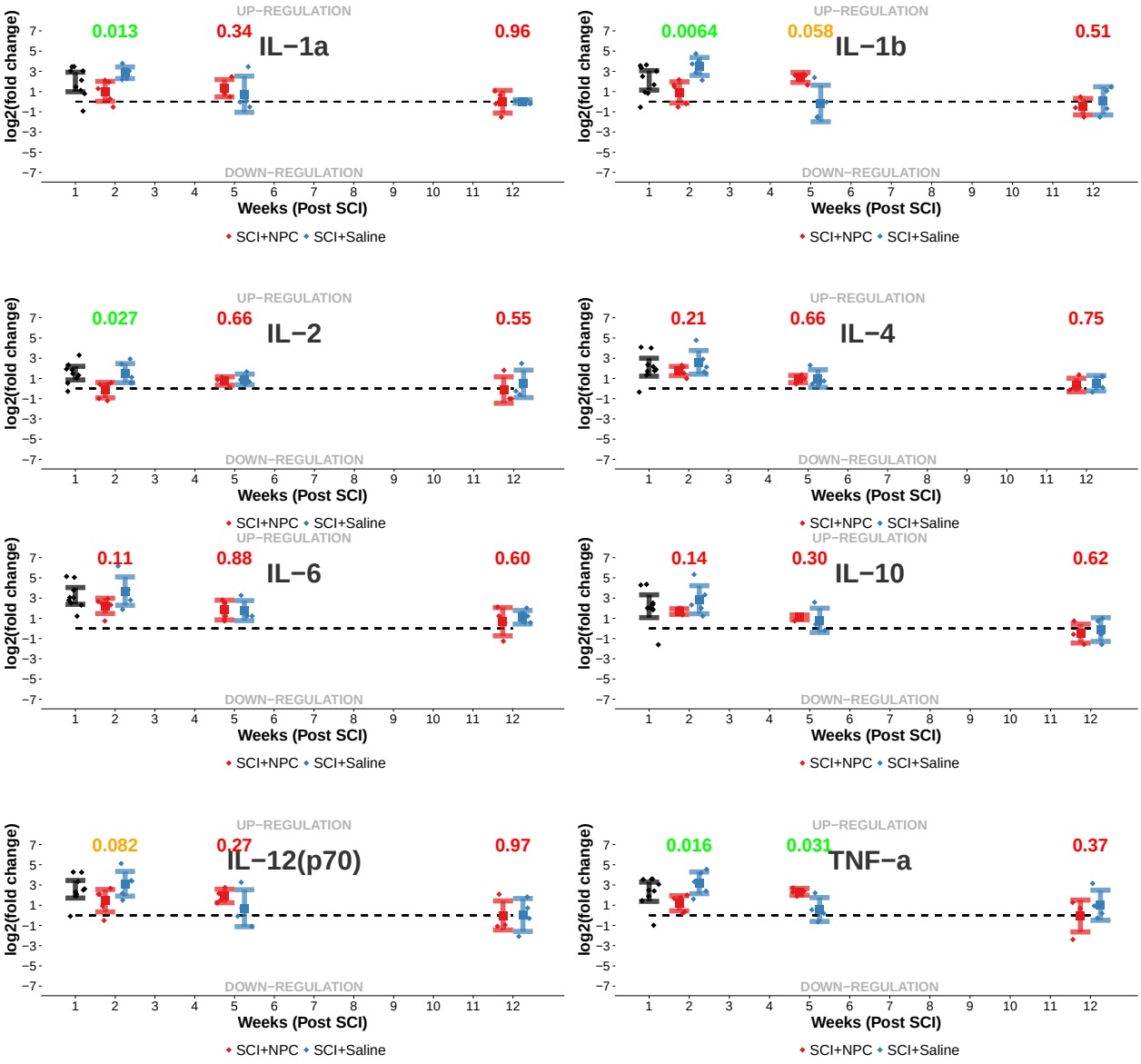
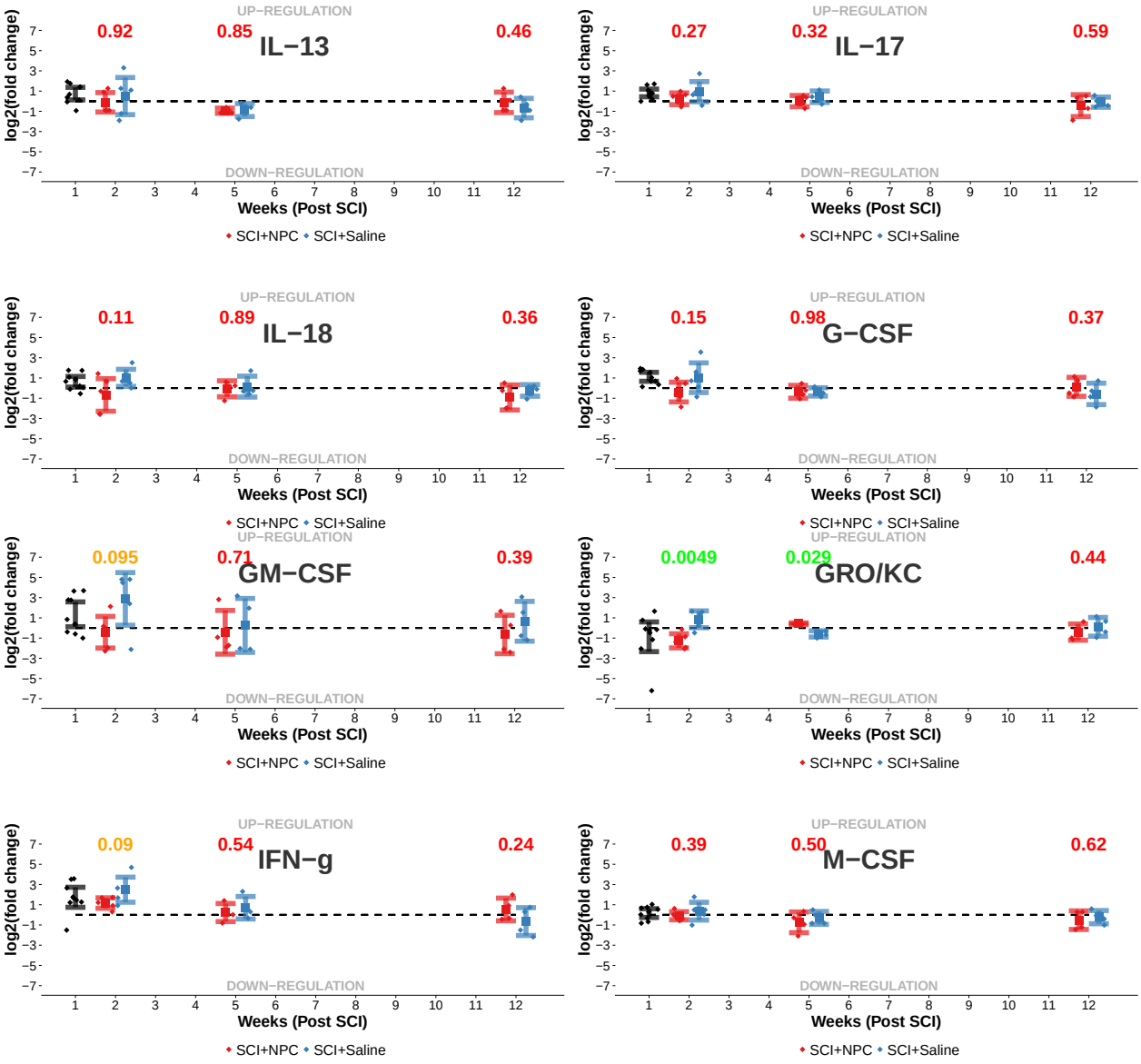


Figure 3: Figure reports agglomerative hierarchical clustering with heatmap of pro-inflammatory cytokines/chemokines for each treatment and time point. Values are log2(fold change in expression in relation to mean expresion in healthy control).

Individual cytokines over time




UP−REGULATION
0.92
0.85
IL−13
0.46
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.27
0.32
IL−17
0.59
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.11
0.89
IL−18
0.36
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.15
0.98
G−CSF
0.37
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.095
0.71
GM−CSF
0.39
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.0049
0.029
GRO/KC
0.44
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.09
0.54
IFN−g
0.24
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline
UP−REGULATION
0.39
0.50
M−CSF
0.62
DOWN−REGULATION
log2(fold change)
Weeks (Post SCI)
SCI+NPC SCI+Saline

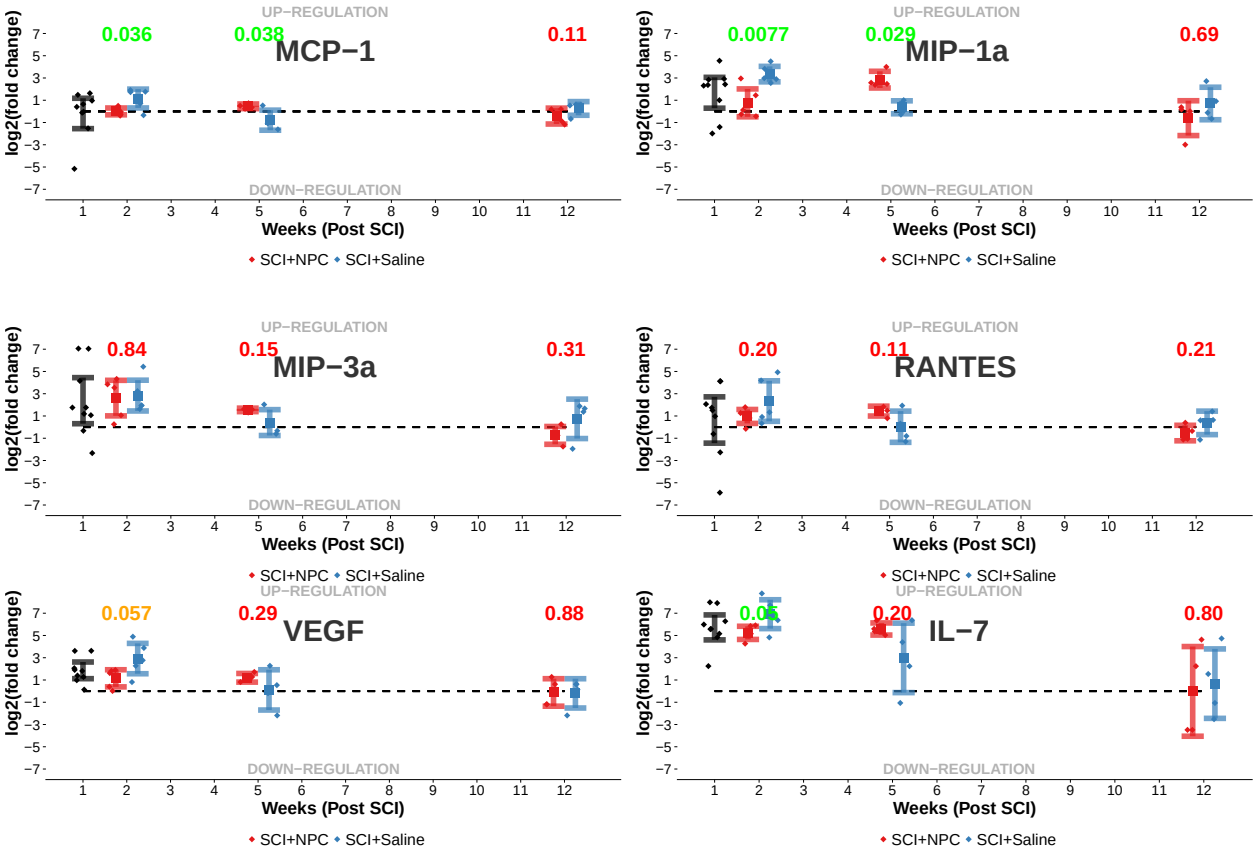


Figure 4: Each plot reports log2(fold change in expression in relation to mean expresion in healthy control) of one cytokine. Statistical analysis as described above. P-values for comparison of the two independent groups are presented at each time point. Color of p-value is green if p-value < 0.05, orange 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.

Week	P-value
2	0.012
5	0.343
12	0.388

Table 2. P-values for mean level of pro-inflammation between treatments within week.

Treatment	P-value
NPC	0.031
saline	0.013

Treatment	5-2	12-2	12-5
NPC	0.623	0.104	0.029
Saline	0.028	0.022	0.989

Table 3. P-values for mean level of pro-inflammation between weeks within treatment.

Target	2w	5w	12w
IL-1a	0.013	0.343	0.956
IL-1b	0.006	0.058	0.508
IL-2	0.027	0.662	0.550
IL-4	0.213	0.655	0.750
IL-5	0.156	0.448	0.484
IL-6	0.110	0.882	0.600
IL-7	0.050	0.202	0.798
IL-10	0.143	0.297	0.624
IL-12(p70)	0.082	0.270	0.966
IL-13	0.916	0.854	0.457
IL-17	0.268	0.324	0.595
IL-18	0.107	0.886	0.355
G-CSF	0.152	0.978	0.370
GM-CSF	0.095	0.711	0.386
GRO/KC	0.005	0.029	0.438
IFN-g	0.090	0.537	0.236
M-CSF	0.387	0.503	0.617
MCP-1	0.036	0.038	0.114
MIP-1a	0.008	0.029	0.686
MIP-3a	0.839	0.150	0.309
RANTES	0.197	0.110	0.207
TNF-a	0.016	0.031	0.373
VEGF	0.057	0.293	0.882

Table 4. P-values for difference in expression of cytokine/chemokine between treatments within week.

Target	NPC	Saline
IL-1a	0.222	0.007
IL-1b	0.004	0.005
IL-2	0.323	0.361
IL-4	0.011	0.035
IL-5	0.127	0.030
IL-6	0.146	0.023
IL-7	0.106	0.016
IL-10	0.001	0.024
IL-12(p70)	0.103	0.039
IL-13	0.351	0.345
IL-17	0.474	0.230
IL-18	0.690	0.152
G-CSF	0.682	0.150
GM-CSF	0.983	0.311
GRO/KC	0.013	0.068
IFN-g	0.305	0.017
M-CSF	0.499	0.430
MCP-1	0.071	0.023
MIP-1a	0.013	0.002
MIP-3a	0.008	0.079
RANTES	0.004	0.119
TNF-a	0.029	0.026
VEGF	0.110	0.026

Table 5. P-values for difference in cytokine/chemokine expression within treatment between week.

```
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
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