Statistical analysis of tissue-scale lifetime ratios

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1 Introduction

In this vignette we present the statistical analysis that was performed on the tissue-scale lifetime ratios in the main paper.

2 Load and inspect data

The data was compiled into a table containing median whole-tissue ratios for each primordium.

> data("statsTable", package="DonaPLLP2013")
> x <- statsTable
> dim(x)
[1] 216  2

> head(x)

        ratio condition
1 0.2923994         WT
2 0.2386834         WT
3 0.1966154         WT
4 0.2129015         WT
5 0.2100342         WT
6 0.1991967         WT

In total we had 6 conditions:

> table(x$condition)

<table>
<thead>
<tr>
<th>Cxcl12a-/-</th>
<th>Cxcr4b-/-</th>
<th>Cxcr7-/-</th>
<th>Cxcr7-/-Cxcl12aMo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>mem-tFT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>46</td>
<td>35</td>
<td>21</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. wild-type (WT),
2. a mutant of the tagged receptor cxcr4b-/ (Cxcr4b-/-),
3. a mutant of the rear ligand-sequestering receptor cxcr7-/ (Cxcr7-/-),
4. a cxcr7-/ mutant with an additional morpholino knockdown of the signalling ligand cxcl12a (Cxcr7-/Cxcl12aMo),
5. a mutant of the signalling ligand cxcl12a, also known as sdf1a (Cxcl12a-/-), and
6. a membrane-tethered control protein tagged with the fluorescent timer (mem-tFT).

> splitByCond <- split(x$ratio, x$condition)
> plotOrder <- c("WT", "Cxcr4b-/-", "Cxcr7-/-", "Cxcr7-/-Cxcl12aMo", "Cxcl12a-/-", + "mem-tFT")
> splitByCond <- splitByCond[plotOrder]
> stripchart(splitByCond, vertical=TRUE, xlab="Condition", ylab="Lifetime Ratio (−)", +
> group.names=1:length(splitByCond))

For 1-5, the readout was the lifetime-ratio from a cxcr4b receptor tagged with the fluorescent timer, which was expressed from a bacterial artificial chromosome. For 6, the readout was the lifetime-ratio from a different, membrane-tethered control protein.

3 Statistical tests

We performed two-sided t-tests for each of the following comparisons of interest.
1. WT to Cxcr4b-/-
2. WT to Cxcr7-/-
3. WT to Cxcl12a-/-
4. WT to mem-tFT
5. Cxcr7-/- to Cxcr7-/-Cxcl12aMo
> compareConds <- as.data.frame(  
+     matrix(nr=6, data=c("WT", "WT", "WT",  
+                  "WT", "Cxcr7-/--", "Cxcr7-/--",  
+                  "Cxcr4b-/--", "Cxcr7-/--", "Cxcl12a-/--",  
+                  "mem-tFT", "Cxcr7-/--Cxcl12aMo", "Cxcr4b-/--"),  
+     stringsAsFactors=FALSE)  
> colnames(compareConds) <- c("condition 1", "condition 2")

Results from the t-tests were appended to our table.

> for (i in seq_len(nrow(compareConds))) {  
+     res <- t.test(x$ratio[x$condition == compareConds[i,1]],  
+                    x$ratio[x$condition == compareConds[i,2]])  
+     compareConds[i, "t"] <- res$statistic  
+     compareConds[i, "df"] <- res$parameter  
+     compareConds[i, "mean 1"] <- res$estimate[1]  
+     compareConds[i, "mean 2"] <- res$estimate[2]  
+     compareConds[i, "difference in means"] <- res$estimate[2]-res$estimate[1]  
+     compareConds[i, "p.value"] <- res$p.value  
+     compareConds[i, "method"] <- res$method  
+ }

> compareConds

<table>
<thead>
<tr>
<th>condition 1</th>
<th>condition 2</th>
<th>t</th>
<th>df</th>
<th>mean 1</th>
<th>mean 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Cxcr4b-/--</td>
<td>4.907150</td>
<td>58.85822</td>
<td>0.3182417</td>
<td>0.2005986</td>
</tr>
<tr>
<td>WT</td>
<td>Cxcr7-/--</td>
<td>9.079875</td>
<td>56.46167</td>
<td>0.3182417</td>
<td>0.1028506</td>
</tr>
<tr>
<td>WT</td>
<td>Cxcl12a-/--</td>
<td>-3.599910</td>
<td>73.09063</td>
<td>0.3182417</td>
<td>0.4389546</td>
</tr>
<tr>
<td>WT</td>
<td>mem-tFT</td>
<td>-11.643242</td>
<td>44.59746</td>
<td>0.3182417</td>
<td>0.9844275</td>
</tr>
<tr>
<td>Cxcr7-/--</td>
<td>Cxcr7-/--Cxcl12aMo</td>
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<td>0.3537685</td>
</tr>
<tr>
<td>Cxcr7-/--</td>
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<td>-7.778590</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>difference in means</th>
<th>p.value</th>
<th>method</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.12071291 5.765433e-04 Welch Two Sample t-test</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.21539114 1.249956e-12 Welch Two Sample t-test</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.66618575 4.098828e-15 Welch Two Sample t-test</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.25091794 3.588092e-10 Welch Two Sample t-test</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.09774801 2.404200e-11 Welch Two Sample t-test</td>
<td></td>
</tr>
</tbody>
</table>

Multiple testing correction was performed using the method of Bonferroni. We noted that since the p-values are so small, this was not a critical step.

> compareConds[, "p.adjusted"] <- p.adjust(compareConds[, "p.value"],  
+ method="bonferroni")  

We preferred to view the table in decreasing order of the change in stability.

> compareConds[order(compareConds[, "condition 1"],  
+               compareConds[, "difference in means"], decreasing=TRUE), ]

<table>
<thead>
<tr>
<th>condition 1</th>
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<th>t</th>
<th>df</th>
<th>mean 1</th>
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</table>

difference in means | p.value | method | p.adjusted |
<table>
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<tr>
<td>4</td>
<td>0.12071291 5.765433e-04 Welch Two Sample t-test</td>
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</tr>
</tbody>
</table>
4 Normality

To assess whether the data were consistent with assumptions of normal distribution, we generated QQ-plots for each condition individually.

```r
> myPlotQQ <- function(residuals, main) {
+     qqnorm(residuals, main=main)
+     qqline(residuals)
+ }
> standardize <- function(x) {(x-mean(x, na.rm=TRUE))/sd(x, na.rm=TRUE)}
> par(mfrow=c(3, 2))
> for (c in unique(x$condition)) {
+     dataPts <- standardize(x[x$condition == c, "ratio"])
+     myPlotQQ(dataPts, c)
+ }
```

![WT QQ-plot](image1)

![Cxcl12a−/− QQ-plot](image2)

![Cxcr4b−/− QQ-plot](image3)

![Cxcr7−/− QQ-plot](image4)

![Cxcr7−/−Cxcl12aMo QQ-plot](image5)

![mem−tFT QQ-plot](image6)
The QQ plots indicated that the data was sufficiently close to being normally distributed.

5 Alternative tests

We also verified that an alternative, non-parametric test, the two-sided Mann-Whitney test (a two-sample Wilcoxon test), returned equivalent results.

```r
> compareCondsMW <- compareConds[, c("condition 1", "condition 2")]
> for (i in seq_len(nrow(compareCondsMW))) {
+   res <- wilcox.test(x$ratio[x$condition == compareCondsMW[i, 1]],
+                        x$ratio[x$condition == compareCondsMW[i, 2]])
+   compareCondsMW[i, "W"] <- res$statistic
+   compareCondsMW[i, "p.value"] <- res$p.value
+   compareCondsMW[i, "method"] <- res$method
+ }
> compareCondsMW

                                 condition 1       condition 2    W       p.value method
1                            WT       Cxcr4b/-/-  1583  7.594851e-06 Wilcoxon rank sum test
2                            WT       Cxcr7/-/-  1515  2.281662e-16 Wilcoxon rank sum test
3                            WT       Cxcl12a/-/-  419  2.695266e-04 Wilcoxon rank sum test
4                            WT       mem-tFT     45  4.265137e-17 Wilcoxon rank sum test
5         Cxcr7/-/-       Cxcr7/-/-Cxcl12aMo  6  4.455117e-14 Wilcoxon rank sum test
6        Cxcr7/-/-       Cxcr4b/-/-  163  2.184994e-11 Wilcoxon rank sum test

We saw that the p-values were extremely similar to those generated by \( t \)-tests. Therefore the biological interpretation of our results was identical in both cases.