Statistical analysis of tissue-scale lifetime ratios

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April 28, 2022

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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
```

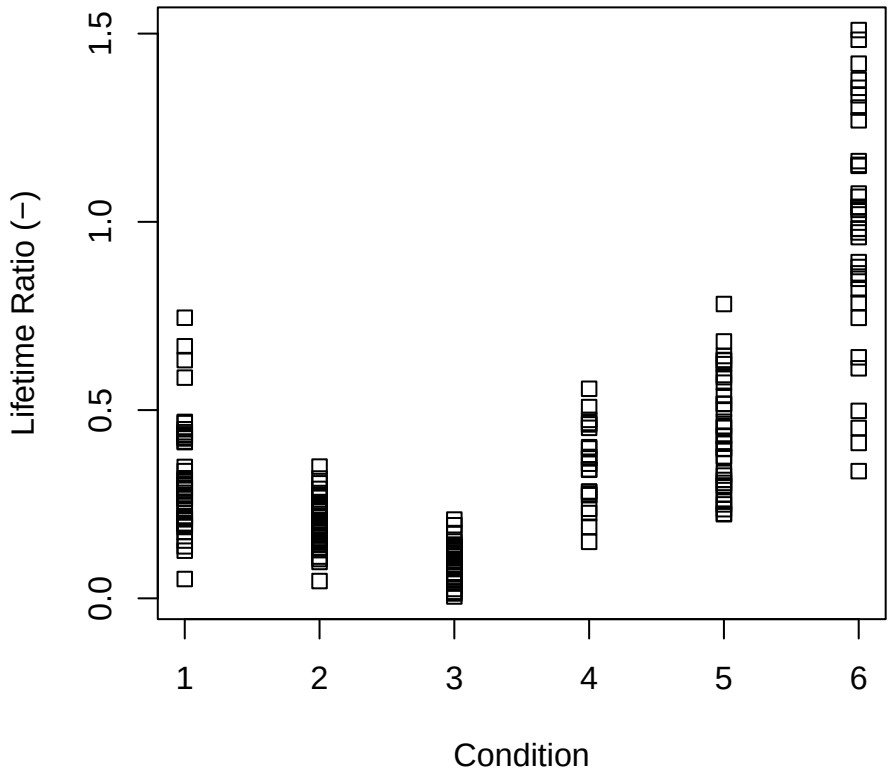
In total we had 6 conditions:

```
> table(x$condition)
```

```
       Cxcl12a-/-         Cxcr4b-/-          Cxcr7-/- Cxcr7-/-Cxcl12aMo
               35                46                35                21
               WT          mem-tFT
               45                34
```

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

6. Cxcr4b-/- to Cxcr7-/-

```
> 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
```

```
  condition 1       condition 2          t       df    mean 1    mean 2
1          WT         Cxcr4b-/-   4.907150 58.85822 0.3182417 0.2005986
2          WT          Cxcr7-/-   9.079875 56.46167 0.3182417 0.1028506
3          WT        Cxcl12a-/-  -3.599910 73.09063 0.3182417 0.4389546
4          WT           mem-tFT -11.643242 44.59746 0.3182417 0.9844275
5    Cxcr7-/- Cxcr7-/-Cxcl12aMo  -9.901493 25.21075 0.1028506 0.3537685
6    Cxcr7-/-         Cxcr4b-/-  -7.778590 78.68026 0.1028506 0.2005986
  difference in means      p.value                  method
1         -0.11764313 7.661584e-06 Welch Two Sample t-test
2         -0.21539114 1.244956e-12 Welch Two Sample t-test
3          0.12071291 5.765433e-04 Welch Two Sample t-test
4          0.66618575 4.098828e-15 Welch Two Sample t-test
5          0.25091794 3.588092e-10 Welch Two Sample t-test
6          0.09774801 2.404200e-11 Welch Two Sample t-test
```

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), ]
```

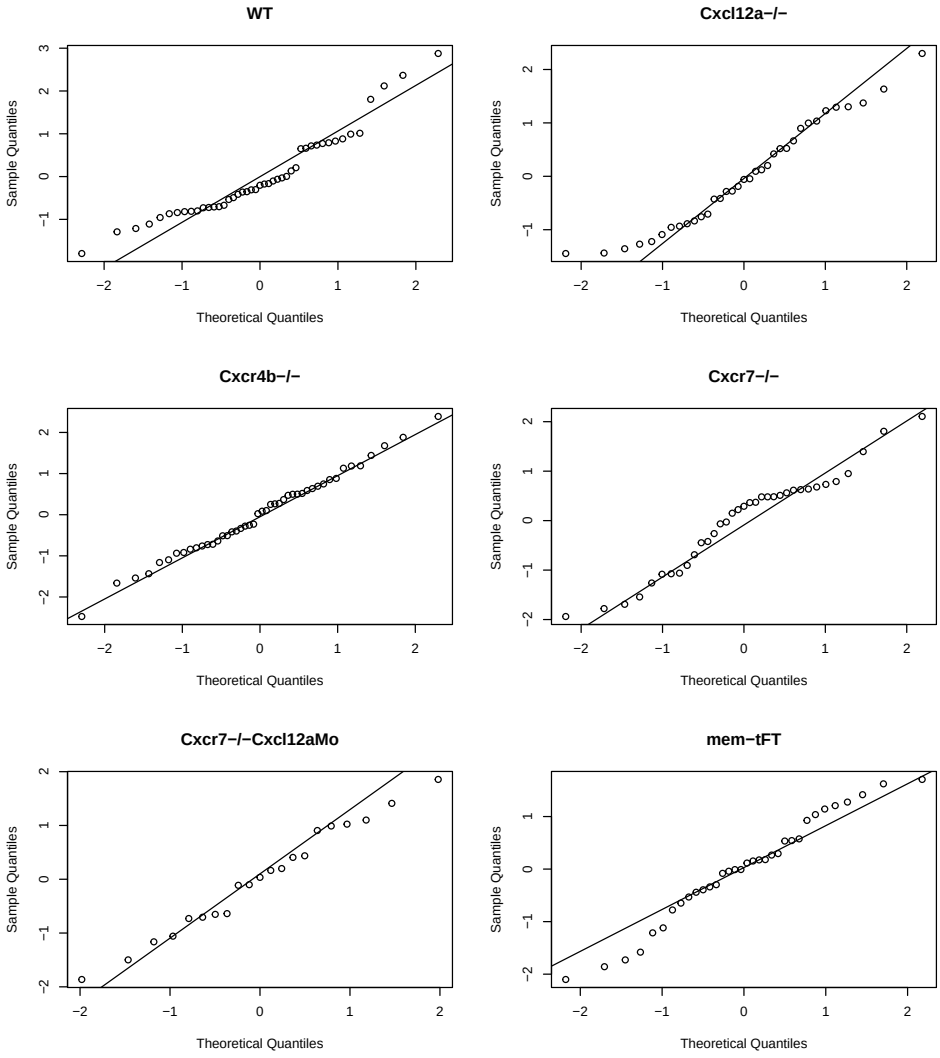
```
  condition 1       condition 2          t       df    mean 1    mean 2
4          WT           mem-tFT -11.643242 44.59746 0.3182417 0.9844275
3          WT        Cxcl12a-/-  -3.599910 73.09063 0.3182417 0.4389546
1          WT         Cxcr4b-/-   4.907150 58.85822 0.3182417 0.2005986
2          WT          Cxcr7-/-   9.079875 56.46167 0.3182417 0.1028506
5    Cxcr7-/- Cxcr7-/-Cxcl12aMo  -9.901493 25.21075 0.1028506 0.3537685
6    Cxcr7-/-         Cxcr4b-/-  -7.778590 78.68026 0.1028506 0.2005986
  difference in means      p.value                  method   p.adjusted
```

```
4          0.66618575 4.098828e-15 Welch Two Sample t-test 2.459297e-14
3          0.12071291 5.765433e-04 Welch Two Sample t-test 3.459260e-03
1         -0.11764313 7.661584e-06 Welch Two Sample t-test 4.596950e-05
2         -0.21539114 1.244956e-12 Welch Two Sample t-test 7.469737e-12
5          0.25091794 3.588092e-10 Welch Two Sample t-test 2.152855e-09
6          0.09774801 2.404200e-11 Welch Two Sample t-test 1.442520e-10
```

4 Normality

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

```
> 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)
+ }
```



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.

```
> 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 exact test
2          WT          Cxcr7-/- 1515 2.281662e-16 Wilcoxon rank sum exact test
3          WT        Cxcl12a-/-  419 2.695266e-04 Wilcoxon rank sum exact test
4          WT           mem-tFT   45 4.265137e-17 Wilcoxon rank sum exact test
5    Cxcr7-/- Cxcr7-/-Cxcl12aMo    6 4.455117e-14 Wilcoxon rank sum exact test
6    Cxcr7-/-         Cxcr4b-/-  163 2.184994e-11 Wilcoxon rank sum exact 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.