

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
```

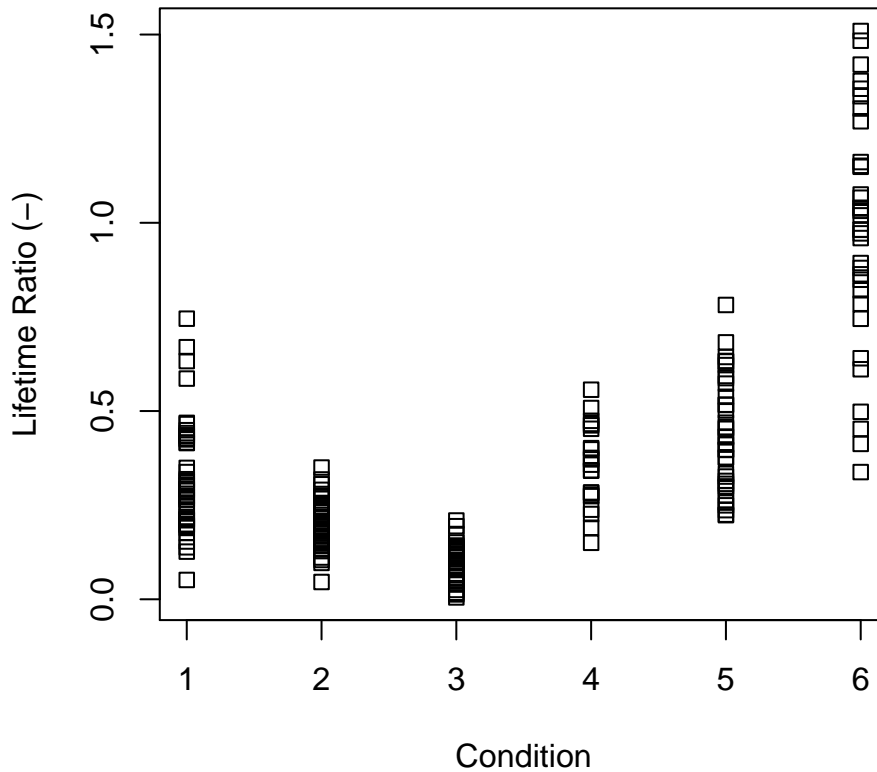
In total we had 6 conditions:

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

```
      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*^{-/-}*Cxcl12a*Mo),
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*^{-/-}*Cxcl12a*Mo

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 | |
| 3 | 0.12071291 | 5.765433e-04 | Welch Two Sample t-test | |
| 1 | -0.11764313 | 7.661584e-06 | Welch Two Sample t-test | |
| 2 | -0.21539114 | 1.244956e-12 | 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 | |

```

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

```

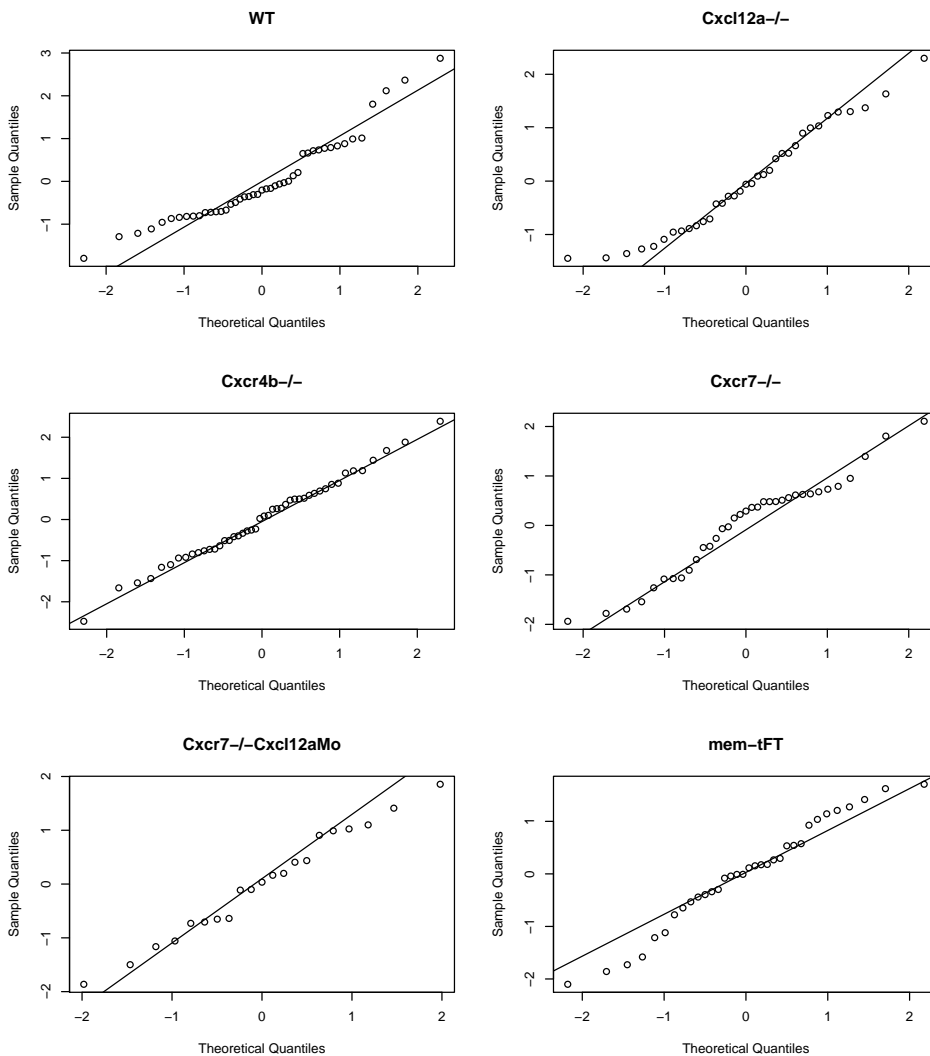
4 Normality

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 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.