

Statistical report: Malnutrition exacerbates pathogenesis of sand fly-transmitted *Leishmania donovani*

Johannes S. P. Doehl

2024-05-22

Summary

This appendix contains the detailed statistical analyses of all the data presented in the main and supplementary figures of the publication titled: “Malnutrition exacerbates pathogenesis of sand fly-transmitted *Leishmania donovani*”. This report was an effort for transparency regarding the applied statistical analyses, in which we also considered retrospective power and sample size calculation to obtain a sense of statistical power in our data to better understand how well our statistical analyses reflected observed biological differences. Insufficient statistical power can result in type II errors, the erroneous acceptance of the null hypothesis that there is no meaningful difference between groups. Even though, we frequently observed lack of statistical power in our data, larger sample sizes were prohibitive due to cost and ethical consideration. That did not detract from the quality of findings presented in the manuscript. On the contrary, observed biological differences were often supported by statistical evaluation and supported the two main observations that infection by sand fly and the nutritional state of the individual had major impacts on the development of leishmaniasis and the infecting parasite, *Leishmania donovani*.

Main Body

General comments

The report is arranged in the order of the figures and their panels as they appear in the main manuscript, to make it easier to accompany. Most data outputs are summarized in tables for easy accessibility. We clearly state the selection process of what we identified as most appropriate comparative analysis for the data for each figure panel. In some cases, data from multiple figure panels were analyzed together as it was one dataset but was split for comprehensive presentation of the data. Also, presented data in the manuscripts figures usually present untransformed datasets, while for the statistical analyses, data transformation was applied as indicated in places. All statistical analyses were performed naive to any expected outcome for unbiased, objective application and interpretation.

Software and packages

All the statistics presented in the manuscript “Malnutrition exacerbates pathogenesis of sand fly-transmitted *Leishmania donovani*” and in this statistical report were produced in RStudio version 2024.4.2.764 [32]. We used R version 4.4.1 [28] and the following R packages: aod v. 1.3.3 [20], betareg v. 3.2.0 [6, 11, 17], bookdown v. 0.40 [37, 38], car v. 3.1.2 [8], caret v. 6.0.94 [18], effectsize v. 0.8.9 [4], emmeans v. 1.10.3 [19], epitools v. 0.5.10.1 [3], ggpubr v. 0.6.0 [14], grid v. 4.4.1 [29], Hmisc v. 5.1.3 [12], janitor v. 2.2.0 [7], knitr v. 1.48 [40, 39, 41], lmtest v. 0.9.40 [44], MASS v. 7.3.61 [34], metan v. 1.18.0 [25], moments v. 0.14.1 [16], multcomp v. 1.4.25 [13], nlme v. 3.1.165 [27, 26], pastecs v. 1.4.2 [10], performance v. 0.12.0 [22], pROC v. 1.18.5 [30], pwrss v. 0.3.1 [5], rcompanion v. 2.4.36 [24], rmarkdown v. 2.27 [42, 43, 2], rstatix v. 0.7.2 [15], sjmisc v. 2.8.10 [21], stringi v. 1.8.4 [9], tidyverse v. 2.0.0 [36], tiff v. 0.1.12 [33], WRS2 v. 1.1.6 [23].¹ For the creation of this statistical report, the author made use of Rmarkdown [1]. The original codes for this statistical report are available as Rmarkdown files through the author’s github portal².

Main Figures

Figure 1

Panel b and c

Data analysis

Figure 1 b and c present the cell counts of total Myeloid_cells and separately, of Neutrophils and Monocytes from $N=60$, 60, 60 pools of single cell suspensions, respectively, prepared from BALB/c mouse ears collected 24 h and 72 h post infection with *Leishmania donovani* by “needle” inoculation or infective sand flies (SF) bites. Please, refer to the methods section of the publication for more details on sample preparation. Note that different mice were sampled at 24 h and 72 h post infection, which meant that this dataset satisfied the independence of data points and therefore, did not represent a repeatedly measured dataset. Thus, this dataset contained three between-subject factors, “Diet” (well-nourished [WN] or malnourished [MN]), “Route” (uninfected control [Ctrl], needle inoculation [Needle], infective sand fly bites [SF]) and “Time_point” (collection at 24 h or 72 h). Based on this information, a three-way analysis was indicated.

Thus, we assessed the data for compliance with assumptions for a three-way ANOVA:

- Data normality
- Homogeneity of variance
- No significant outliers

Initial assumption assessment indicated that data transformation was required to meet assumptions for a

¹R package citations were managed using the ‘grateful’ package [31], while inter-package function name conflicts were managed with the ‘conflicted’ package [35]

²<https://github.com/joedoehl/Malnutrition-exacerbates-pathogenesis-of-sand-fly-transmitted-Leishmania-donovani.git>

three-way ANOVA. Thus, we settled for a Box-Cox power transformation of all datasets presented in this figure. Thus, data distribution and variance appear different in the main figure panels in the publication from the once that were used in the analysis post transformation.

Assumption analyses

Data normality

The assessment of the transformed data distribution for each group was conducted by Shpiro-Wilks test and QQ-plot for counts of Myeloid_cells, Neutrophils and Monocytes separately. Note that all groups of all datasets consisted of $N=5$ pools of mouse ear single-cell suspensions, which made it difficult to assess data distribution reliably by Shapiro-Wilks test.

Myeloid_cells

In spite of assessment limitations due to small group sizes, we concluded based on the Shapiro-Wilks test (Appendix table 1) and QQ-plots (Fig.1b-c-1) that all groups of the dataset were likely to follow a normal distribution.

Appendix Table 1
Myeloid cells: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Ctrl | 24_h | Counts | 0.9919 | 0.9860 | ns |
| WN | Ctrl | 72_h | Counts | 0.9919 | 0.9860 | ns |
| MN | Ctrl | 24_h | Counts | 0.9378 | 0.6502 | ns |
| MN | Ctrl | 72_h | Counts | 0.9378 | 0.6502 | ns |
| WN | Needle | 24_h | Counts | 0.8543 | 0.2084 | ns |
| WN | Needle | 72_h | Counts | 0.9022 | 0.4219 | ns |
| MN | Needle | 24_h | Counts | 0.8424 | 0.1716 | ns |
| MN | Needle | 72_h | Counts | 0.9791 | 0.9298 | ns |
| WN | SF | 24_h | Counts | 0.7891 | 0.0659 | ns |
| WN | SF | 72_h | Counts | 0.7877 | 0.0641 | ns |
| MN | SF | 24_h | Counts | 0.9207 | 0.5344 | ns |
| MN | SF | 72_h | Counts | 0.9947 | 0.9933 | ns |

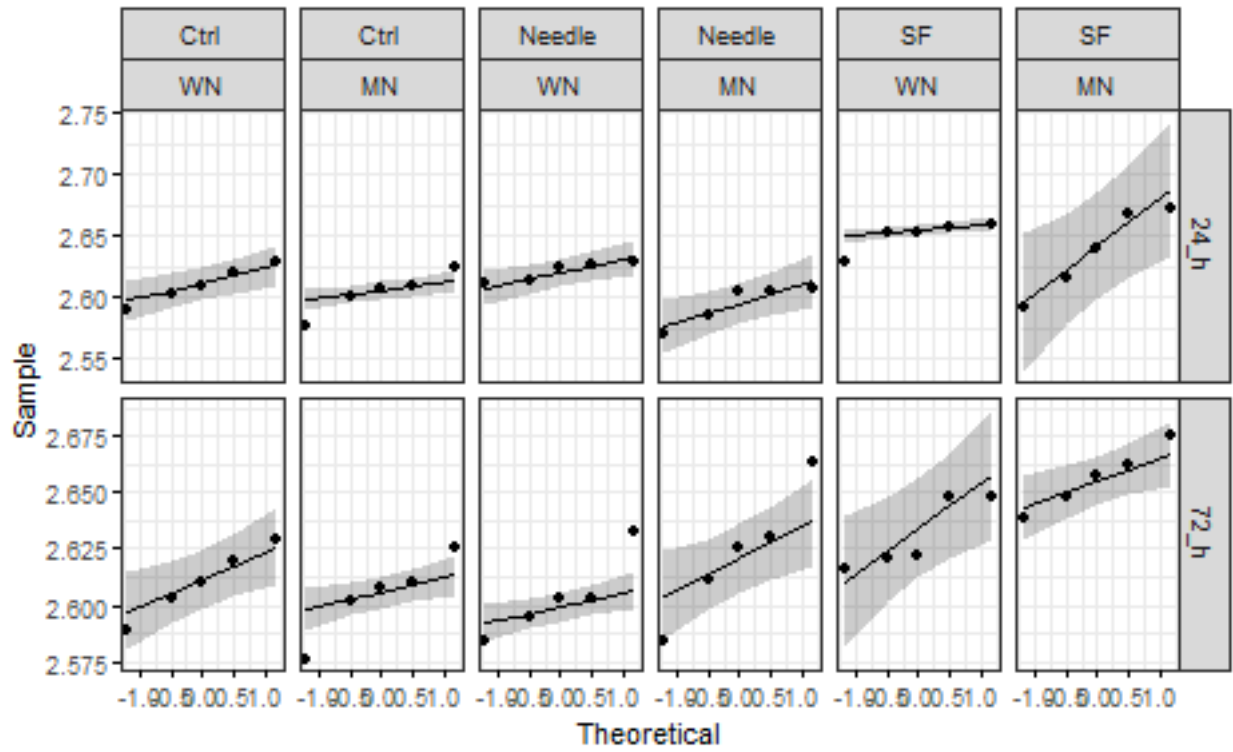


Fig.1b-c-1: QQ-plots of myeloid cell counts split into groups by predictor variables

Neutrophils

In spite of assessment limitations due to small group sizes, we concluded based on the Shapiro-Wilks test (Appendix table 2) and QQ-plots (Fig.1b-c-2) that all groups of the dataset were likely to follow a normal distribution.

Appendix Table 2
Neutrophils: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Ctrl | 24_h | Counts | 0.9133 | 0.4879 | ns |
| WN | Ctrl | 72_h | Counts | 0.9133 | 0.4879 | ns |
| MN | Ctrl | 24_h | Counts | 0.9609 | 0.8144 | ns |
| MN | Ctrl | 72_h | Counts | 0.9609 | 0.8144 | ns |
| WN | Needle | 24_h | Counts | 0.8948 | 0.3818 | ns |
| WN | Needle | 72_h | Counts | 0.9849 | 0.9590 | ns |
| MN | Needle | 24_h | Counts | 0.8150 | 0.1068 | ns |
| MN | Needle | 72_h | Counts | 0.9745 | 0.9032 | ns |
| WN | SF | 24_h | Counts | 0.7958 | 0.0748 | ns |
| WN | SF | 72_h | Counts | 0.9210 | 0.5361 | ns |
| MN | SF | 24_h | Counts | 0.8692 | 0.2632 | ns |

| | | | | | | |
|----|----|------|--------|--------|--------|----|
| MN | SF | 72_h | Counts | 0.9840 | 0.9547 | ns |
|----|----|------|--------|--------|--------|----|

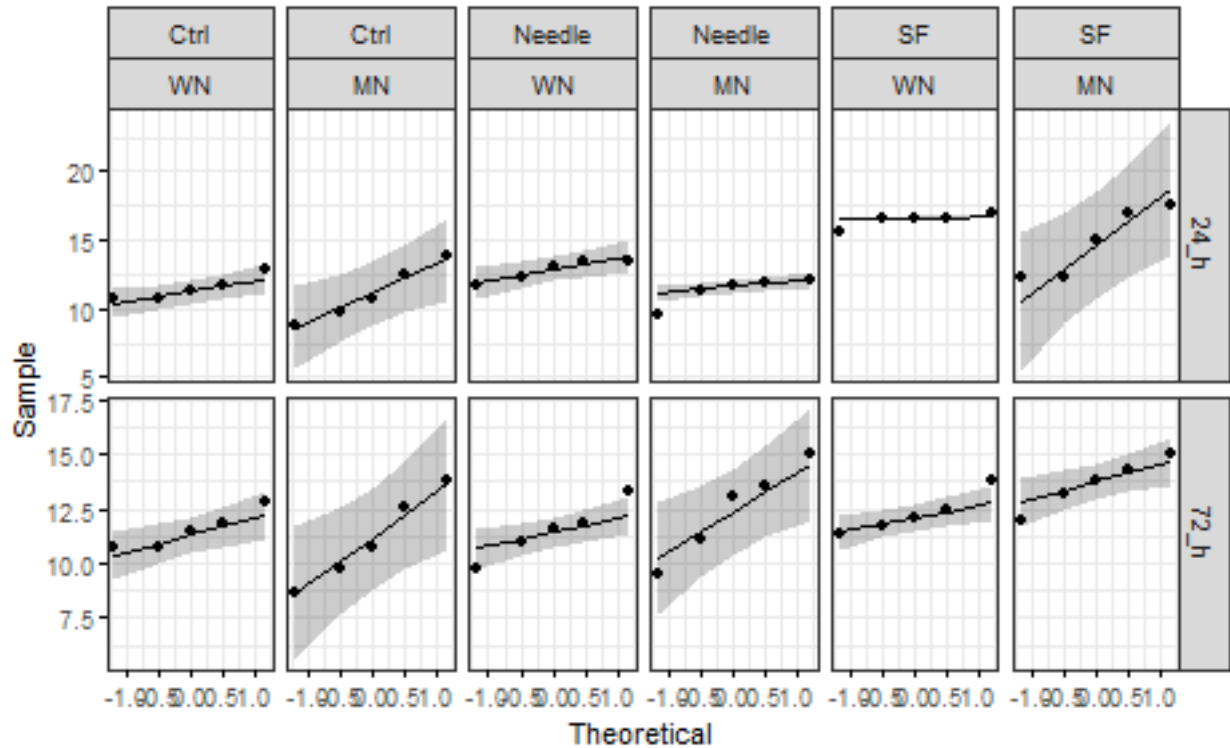


Fig.1b-c-2: QQ-plots of neutrophil counts split into groups by predictor variables

Monocytes

In spite of assessment limitations due to small group sizes, we concluded based on the Shapiro-Wilks test (Appendix table 3) and QQ-plots (Fig.1b-c-3) that all groups of the dataset were likely to follow a normal distribution.

Appendix Table 3
Monocytes: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Ctrl | 24_h | Counts | 0.8597 | 0.2271 | ns |
| WN | Ctrl | 72_h | Counts | 0.8597 | 0.2271 | ns |
| MN | Ctrl | 24_h | Counts | 0.8517 | 0.1999 | ns |
| MN | Ctrl | 72_h | Counts | 0.8517 | 0.1999 | ns |
| WN | Needle | 24_h | Counts | 0.8945 | 0.3803 | ns |
| WN | Needle | 72_h | Counts | 0.9625 | 0.8250 | ns |
| MN | Needle | 24_h | Counts | 0.9079 | 0.4552 | ns |
| MN | Needle | 72_h | Counts | 0.9435 | 0.6907 | ns |
| WN | SF | 24_h | Counts | 0.9944 | 0.9927 | ns |

| | | | | | | |
|----|----|------|--------|--------|--------|----|
| WN | SF | 72_h | Counts | 0.8455 | 0.1806 | ns |
| MN | SF | 24_h | Counts | 0.9226 | 0.5466 | ns |
| MN | SF | 72_h | Counts | 0.9595 | 0.8048 | ns |

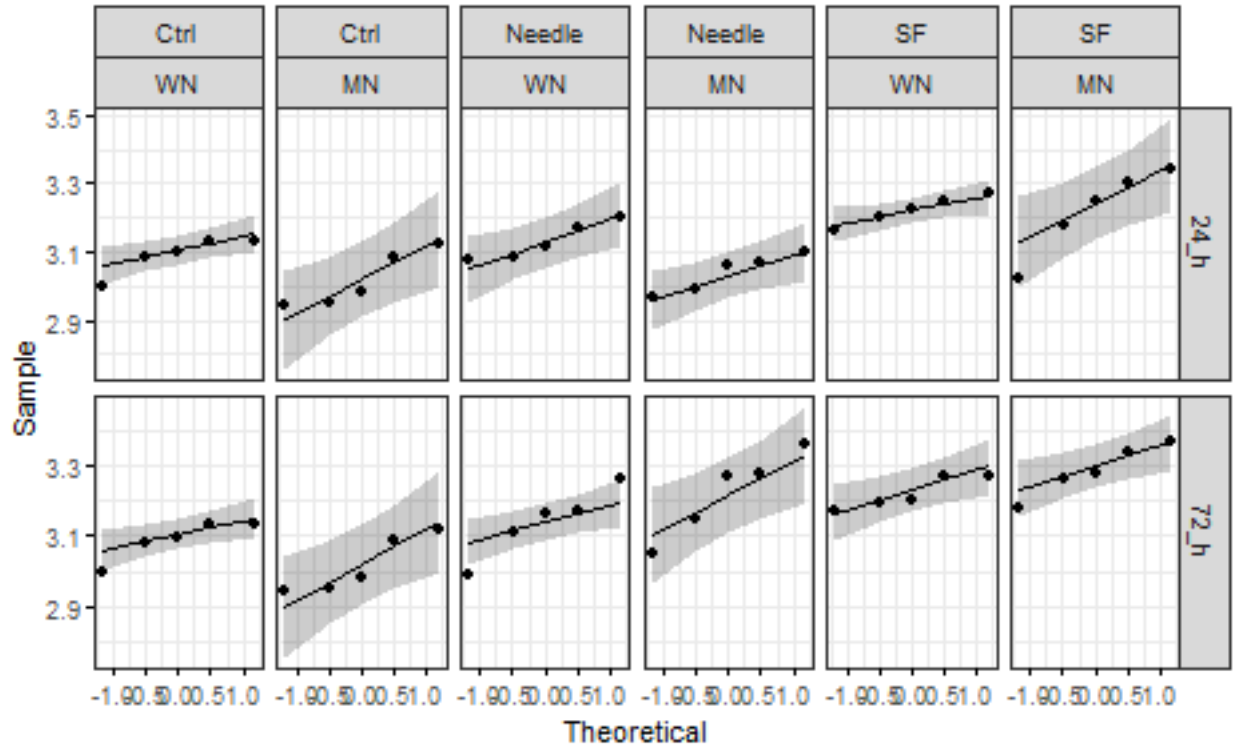


Fig.1b-c-3: QQ-plots of monocyte counts split into groups by predictor variables

Homogeneity of variance The assessment of homogeneity of variance was also conducted for myeloid cell, neutrophil and monocyte counts separately. We employed the Levene’s test on each dataset. The p-value for the test suggested that the assumption of homogeneity of variance held for Myeloid_cells (p=0.5236), held for Neutrophils (p=0.0502), and held for Monocytes (p=0.7728).

Outliers It can be difficult to determine outliers in small datasets reliably as the analysis is dependent on the interquartile range of the data per group. We attempted it anyway and found a potential 8 outliers in the Myeloid_cells dataset (Appendix table 4), 5 outliers in the Neutrophils dataset (Appendix table 5), and 3 outliers in the Monocytes dataset (Appendix table 6), of which some were classified as extreme. However, larger datasets may have found these not be outliers, but just peripheral.

Appendix Table 4
Myeloid cells: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|-------|------------|------------|------------|
| MN | Ctrl | 24_h | TRUE | FALSE |

| | | | | |
|----|--------|------|------|-------|
| MN | Ctrl | 24_h | TRUE | FALSE |
| MN | Ctrl | 72_h | TRUE | FALSE |
| MN | Ctrl | 72_h | TRUE | FALSE |
| WN | Needle | 72_h | TRUE | TRUE |
| MN | Needle | 72_h | TRUE | FALSE |
| MN | Needle | 72_h | TRUE | FALSE |
| WN | SF | 24_h | TRUE | TRUE |

Appendix Table 5
Neutrophils: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|--------|------------|------------|------------|
| WN | Needle | 72_h | TRUE | FALSE |
| MN | Needle | 24_h | TRUE | TRUE |
| WN | SF | 24_h | TRUE | TRUE |
| WN | SF | 24_h | TRUE | FALSE |
| WN | SF | 72_h | TRUE | FALSE |

Appendix Table 6
Monocytes: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|--------|------------|------------|------------|
| WN | Ctrl | 24_h | TRUE | FALSE |
| WN | Ctrl | 72_h | TRUE | FALSE |
| WN | Needle | 72_h | TRUE | FALSE |

Three-way analysis

The appropriate three-way analysis was performed for Myeloid_cells, Neutrophils and Monocytes separately.

Myeloid_cells

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the Myeloid_cells dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Myeloid_cells counts per pooled ear single-cell suspension (Appendix table 7). The test output showed that only the infection “Route” was a statistically significant predictor, while the interaction between Time_point and Diet was statistically significant, too.

Appendix Table 7
Myeloid cells: Robust three-way ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|---------|---------|------|
| Diet | 0.0048 | 0.9500 | ns |
| Route | 29.5689 | 0.0007 | *** |
| Time_point | 0.0447 | 0.8390 | ns |
| Diet:Route | 0.7681 | 0.7120 | ns |
| Diet:Time_point | 7.4482 | 0.0230 | * |
| Route:Time_point | 0.2226 | 0.9040 | ns |
| Diet:Route:Time_point | 7.4542 | 0.0720 | + |

We looked for main effects by splitting the data by each predictor separately and analyzed the respective remaining two predictor by a Robust two-way ANOVA. The results showed that, after adjustment of p-values for multiple tests, only the “Route” predictor produced statistically significant p-values, regardless of whether the data was split by “Diet” or “Time-point” (Appendix table 8). This suggested that the only predictor of impact on Myeloid_cells counts was the route of infection.

Appendix Table 8
Myeloid cells: Robust two-way ANOVA

| Grouper | Predictor | value | p.value | Sig. | p.value.adj | sig. |
|------------------|------------------|---------|---------|------|-------------|------|
| Grouped by Route | | | | | | |
| Ctrl | Diet | 0.7025 | 0.415 | ns | 0.5810 | ns |
| Ctrl | Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Ctrl | Diet:Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Needle | Diet | 0.1500 | 0.707 | ns | 0.8248 | ns |
| Needle | Time_point | 0.4370 | 0.523 | ns | 0.6510 | ns |
| Needle | Diet:Time_point | 6.7428 | 0.024 | * | 0.1008 | ns |
| SF | Diet | 0.4354 | 0.527 | ns | 0.6510 | ns |
| SF | Time_point | 0.0015 | 0.971 | ns | 0.9990 | ns |
| SF | Diet:Time_point | 4.1861 | 0.069 | + | 0.1701 | ns |
| Grouped by Diet | | | | | | |
| WN | Route | 28.0464 | 0.001 | *** | 0.0105 | * |
| WN | Time_point | 5.2407 | 0.032 | * | 0.1120 | ns |
| WN | Route:Time_point | 2.5384 | 0.326 | ns | 0.5444 | ns |
| MN | Route | 19.2056 | 0.003 | ** | 0.0210 | * |
| MN | Time_point | 3.5769 | 0.076 | + | 0.1701 | ns |
| MN | Route:Time_point | 2.5075 | 0.337 | ns | 0.5444 | ns |

Grouped by Time_point

| | | | | | | |
|------|------------|---------|-------|-----|--------|----|
| 24_h | Diet | 4.6264 | 0.050 | * | 0.1500 | ns |
| 24_h | Route | 17.3213 | 0.004 | ** | 0.0210 | * |
| 24_h | Diet:Route | 2.3091 | 0.368 | ns | 0.5520 | ns |
| 72_h | Diet | 3.3991 | 0.081 | + | 0.1701 | ns |
| 72_h | Route | 29.3554 | 0.001 | *** | 0.0105 | * |
| 72_h | Diet:Route | 5.2718 | 0.112 | ns | 0.2138 | ns |

For the analysis of the simple simple main effect for each respective predictor, we performed Robust one-way ANOVA with individual predictors of the data split by the other two predictors. The output showed yet again that after the adjustment of p-values for multiple tests, only the “Route” predictor produce statistically significant p-values (Appendix table 9). Here, the differences were observed in the well-nourished group (WN) at 24 h post infection and for the malnourished group at 72 h post infection.

Appendix Table 9
Myeloid cells: Robust one-way ANOVA

| Predictor_1 | Predictor_2 | Effect | test | df1 | df2 | p.value | effsize | p.value.adj | sig. |
|-----------------------|-------------|------------|---------|-----|--------|---------|---------|-------------|------|
| Predictor: Time_point | | | | | | | | | |
| Ctrl | WN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Ctrl | MN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Needle | WN | Time_point | 3.5282 | 1 | 5.5647 | 0.1132 | 0.7337 | 0.2033 | ns |
| Needle | MN | Time_point | 3.6121 | 1 | 6.2598 | 0.1041 | 0.9017 | 0.2033 | ns |
| SF | WN | Time_point | 4.8246 | 1 | 7.5253 | 0.0614 | 0.7261 | 0.1965 | ns |
| SF | MN | Time_point | 1.3003 | 1 | 5.2694 | 0.3033 | 0.5259 | 0.4412 | ns |
| Predictor: Diet | | | | | | | | | |
| 24_h | Ctrl | Diet | 0.3512 | 1 | 7.8195 | 0.5702 | 0.2800 | 0.6516 | ns |
| 24_h | Needle | Diet | 10.1859 | 1 | 5.9402 | 0.0191 | 0.9926 | 0.1017 | ns |
| 24_h | SF | Diet | 0.6400 | 1 | 4.9962 | 0.4600 | 0.3842 | 0.6134 | ns |
| 72_h | Ctrl | Diet | 0.3512 | 1 | 7.8195 | 0.5702 | 0.2800 | 0.6516 | ns |
| 72_h | Needle | Diet | 1.5616 | 1 | 6.7245 | 0.2532 | 0.5753 | 0.4051 | ns |
| 72_h | SF | Diet | 7.3397 | 1 | 7.8679 | 0.0271 | 0.8992 | 0.1084 | ns |
| Predictor: Route | | | | | | | | | |
| 24_h | WN | Route | 12.5661 | 2 | 7.4933 | 0.0040 | 0.9018 | 0.0323 | * |
| 24_h | MN | Route | 2.9293 | 2 | 7.5513 | 0.1144 | 0.6507 | 0.2033 | ns |
| 72_h | WN | Route | 3.3900 | 2 | 7.9569 | 0.0861 | 0.6194 | 0.2033 | ns |
| 72_h | MN | Route | 12.7031 | 2 | 7.4981 | 0.0039 | 0.7858 | 0.0323 | * |

For the pairwise comparison, we applied a Linear contrast expression. The output showed that at 24 h post

infection, the sand fly infection route was statistically significantly different from the control and needle inoculum in the well-nourished (WN) group (Appendix table 10). At 72 h, the sand fly infection route was statistically significantly different from the control in the malnourished group. Further, statistically significant difference were observed between well-nourished and malnourished mice inoculated by needle at 24 h post infection and infected by sand fly at 72 h post infection.

Appendix Table 10
Myeloid cells: Pairwise comparison by Linear Contrast Expression

| Predictor_1 | Predictor_2 | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------------------|-------------|--------|---------|---------|----------|----------|---------|------|
| Predictor: Time_point | | | | | | | | |
| Ctrl | WN | 24_h | 72_h | 0.0000 | -0.0224 | 0.0224 | 1.0000 | ns |
| Ctrl | MN | 24_h | 72_h | 0.0000 | -0.0261 | 0.0261 | 1.0000 | ns |
| Needle | WN | 24_h | 72_h | 0.0169 | -0.0055 | 0.0394 | 0.1132 | ns |
| Needle | MN | 24_h | 72_h | -0.0285 | -0.0647 | 0.0078 | 0.1041 | ns |
| SF | WN | 24_h | 72_h | 0.0197 | -0.0012 | 0.0406 | 0.0614 | + |
| SF | MN | 24_h | 72_h | -0.0190 | -0.0611 | 0.0231 | 0.3033 | ns |
| Predictor: Diet | | | | | | | | |
| 24_h | Ctrl | WN | MN | 0.0062 | -0.0182 | 0.0306 | 0.5702 | ns |
| 24_h | Needle | WN | MN | 0.0261 | 0.0060 | 0.0461 | 0.0191 | * |
| 24_h | SF | WN | MN | 0.0131 | -0.0290 | 0.0552 | 0.4600 | ns |
| 72_h | Ctrl | WN | MN | 0.0062 | -0.0182 | 0.0306 | 0.5702 | ns |
| 72_h | Needle | WN | MN | -0.0193 | -0.0561 | 0.0175 | 0.2532 | ns |
| 72_h | SF | WN | MN | -0.0256 | -0.0474 | -0.0037 | 0.0271 | * |
| Predictor: Route | | | | | | | | |
| 24_h | WN | Ctrl | Needle | -0.0103 | -0.0350 | 0.0144 | 0.2350 | ns |
| 24_h | WN | Ctrl | SF | -0.0403 | -0.0666 | -0.0140 | 0.0040 | ** |
| 24_h | WN | Needle | SF | -0.0300 | -0.0502 | -0.0098 | 0.0040 | ** |
| 24_h | MN | Ctrl | Needle | 0.0096 | -0.0225 | 0.0416 | 0.4028 | ns |
| 24_h | MN | Ctrl | SF | -0.0334 | -0.0888 | 0.0220 | 0.1542 | ns |
| 24_h | MN | Needle | SF | -0.0430 | -0.0983 | 0.0123 | 0.1421 | ns |
| 72_h | WN | Ctrl | Needle | 0.0066 | -0.0253 | 0.0385 | 0.5523 | ns |
| 72_h | WN | Ctrl | SF | -0.0206 | -0.0498 | 0.0087 | 0.1060 | ns |
| 72_h | WN | Needle | SF | -0.0272 | -0.0595 | 0.0050 | 0.1060 | ns |
| 72_h | MN | Ctrl | Needle | -0.0189 | -0.0665 | 0.0287 | 0.2597 | ns |
| 72_h | MN | Ctrl | SF | -0.0524 | -0.0828 | -0.0219 | 0.0031 | ** |
| 72_h | MN | Needle | SF | -0.0335 | -0.0804 | 0.0135 | 0.0936 | + |

Neutrophils

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the Neutrophils dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Neutrophils count per pooled ear single-cell suspension (Appendix table 11). As for Myeloid_cells, the test output showed that only the infection “Route” was a statistically significant predictor, but none of the interaction terms were.

Appendix Table 11
Neutrophils: Robust three-way ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|---------|---------|------|
| Diet | 0.1394 | 0.720 | ns |
| Route | 23.0377 | 0.004 | ** |
| Time_point | 4.3763 | 0.065 | + |
| Diet:Route | 0.0479 | 0.979 | ns |
| Diet:Time_point | 4.0582 | 0.074 | + |
| Route:Time_point | 5.4366 | 0.150 | ns |
| Diet:Route:Time_point | 1.9427 | 0.458 | ns |

We looked for main effects by splitting the data by each predictor separately and analyzed the respective remaining two predictor by a Robust two-way ANOVA. After adjustment of p-values for multiple tests, we observed statistically significant differences between 24 h and 72 h post infection, when the data was split by infestation route, in the well-nourished group for Route, Time_point and their interaction, as much as for infection route in the malnourished group, when the data was split by “Diet”; and for infection routes at 24 h post infection, when the data was split by Time_point (Appendix table 12).

Appendix Table 12
Neutrophils: Robust two-way ANOVA

| Grouper | Predictor | value | p.value | Sig. | p.value.adj | sig. |
|------------------|-----------------|---------|---------|------|-------------|------|
| Grouped by Route | | | | | | |
| Ctrl | Diet | 0.3205 | 0.583 | ns | 0.8745 | ns |
| Ctrl | Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Ctrl | Diet:Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Needle | Diet | 0.1839 | 0.678 | ns | 0.8899 | ns |
| Needle | Time_point | 0.0428 | 0.841 | ns | 0.9812 | ns |
| Needle | Diet:Time_point | 3.7795 | 0.078 | + | 0.1638 | ns |
| SF | Diet | 0.0473 | 0.834 | ns | 0.9812 | ns |
| SF | Time_point | 16.1728 | 0.003 | ** | 0.0126 | * |
| SF | Diet:Time_point | 5.0287 | 0.053 | + | 0.1237 | ns |

| Grouped by Diet | | | | | | |
|-----------------------|------------------|---------|-------|-----|--------|----|
| WN | Route | 68.7607 | 0.001 | *** | 0.0053 | ** |
| WN | Time_point | 31.4785 | 0.001 | *** | 0.0053 | ** |
| WN | Route:Time_point | 34.6536 | 0.001 | *** | 0.0053 | ** |
| MN | Route | 13.4578 | 0.009 | ** | 0.0315 | * |
| MN | Time_point | 0.0021 | 0.964 | ns | 0.9990 | ns |
| MN | Route:Time_point | 1.9351 | 0.423 | ns | 0.6833 | ns |
| Grouped by Time_point | | | | | | |
| 24_h | Diet | 4.6992 | 0.050 | * | 0.1237 | ns |
| 24_h | Route | 37.4309 | 0.001 | *** | 0.0053 | ** |
| 24_h | Diet:Route | 0.9951 | 0.644 | ns | 0.8899 | ns |
| 72_h | Diet | 1.3219 | 0.267 | ns | 0.5097 | ns |
| 72_h | Route | 8.1667 | 0.046 | * | 0.1237 | ns |
| 72_h | Diet:Route | 2.1155 | 0.395 | ns | 0.6833 | ns |

For the analysis of the simple simple main effect for each respective predictor, we performed Robust one-way ANOVA with individual predictors of the data split by the other two predictors. The output showed statistically significant p-values for the sand fly inoculation in the well-nourished group for the Time_point predictor and at 24 h post infection in the well-nourished group for the infection route (Appendix table 13).

Appendix Table 13
Neutrophils: Robust one-way ANOVA

| Predictor_1 | Predictor_2 | Effect | test | df1 | df2 | p.value | effsize | p.value.adj | sig. |
|-----------------------|-------------|------------|---------|-----|--------|---------|---------|-------------|------|
| Predictor: Time_point | | | | | | | | | |
| Ctrl | WN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Ctrl | MN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Needle | WN | Time_point | 4.1593 | 1 | 6.4828 | 0.0840 | 0.8107 | 0.2240 | ns |
| Needle | MN | Time_point | 1.0452 | 1 | 5.6120 | 0.3486 | 0.4543 | 0.5706 | ns |
| SF | WN | Time_point | 76.4341 | 1 | 5.6989 | 0.0002 | 1.0898 | 0.0013 | ** |
| SF | MN | Time_point | 0.9078 | 1 | 5.5609 | 0.3803 | 0.3810 | 0.5706 | ns |
| Predictor: Diet | | | | | | | | | |
| 24_h | Ctrl | Diet | 0.1603 | 1 | 5.3717 | 0.7043 | 0.1989 | 0.8050 | ns |
| 24_h | Needle | Diet | 7.1359 | 1 | 7.4476 | 0.0302 | 1.0777 | 0.1609 | ns |
| 24_h | SF | Diet | 2.0228 | 1 | 4.2581 | 0.2239 | 0.5190 | 0.4478 | ns |
| 72_h | Ctrl | Diet | 0.1603 | 1 | 5.3717 | 0.7043 | 0.1989 | 0.8050 | ns |
| 72_h | Needle | Diet | 0.7151 | 1 | 6.4714 | 0.4279 | 0.3795 | 0.5706 | ns |

| | | | | | | | | | |
|------------------|----|-------|---------|---|--------|---------|--------|--------|-----|
| 72_h | SF | Diet | 4.0661 | 1 | 7.7816 | 0.0795 | 0.7530 | 0.2240 | ns |
| Predictor: Route | | | | | | | | | |
| 24_h | WN | Route | 78.7826 | 2 | 7.3614 | <0.0001 | 0.9442 | 0.0002 | *** |
| 24_h | MN | Route | 3.9055 | 2 | 6.8243 | 0.0740 | 0.7267 | 0.2240 | ns |
| 72_h | WN | Route | 0.9855 | 2 | 7.8365 | 0.4152 | 0.4338 | 0.5706 | ns |
| 72_h | MN | Route | 2.7786 | 2 | 7.2716 | 0.1269 | 0.5443 | 0.2901 | ns |

For the pairwise comparison, we applied a Linear contrast expression. The output showed that there were statistically significant differences between time point for well-nourished, sand fly inoculated mice, at 24 h post infection between the well-nourished and malnourished groups for the needle inoculum and at 72 h post infection for the sand fly inoculation (Appendix table 14). Further, we observed differences in the well-nourished group at 24 h post infection between the sand fly inoculum and both, the control and needle inoculum. For the malnourished group we observed statistically significant p-values at 72 h post infection between the sand fly inoculum and the control group.

Appendix Table 14
Neutrophils: Pairwise comparison by Linear Contrast Expression

| Predictor_1 | Predictor_2 | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------------------|-------------|--------|---------|---------|----------|----------|---------|------|
| Predictor: Time_point | | | | | | | | |
| Ctrl | WN | 24_h | 72_h | 0.0000 | -1.2773 | 1.2773 | 1.0000 | ns |
| Ctrl | MN | 24_h | 72_h | 0.0000 | -3.0375 | 3.0375 | 1.0000 | ns |
| Needle | WN | 24_h | 72_h | 1.3539 | -0.2416 | 2.9494 | 0.0840 | + |
| Needle | MN | 24_h | 72_h | -1.0936 | -3.7554 | 1.5683 | 0.3486 | ns |
| SF | WN | 24_h | 72_h | 4.1934 | 3.0045 | 5.3822 | 0.0002 | *** |
| SF | MN | 24_h | 72_h | 1.1910 | -1.9272 | 4.3091 | 0.3803 | ns |
| Predictor: Diet | | | | | | | | |
| 24_h | Ctrl | WN | MN | 0.4045 | -2.1397 | 2.9487 | 0.7043 | ns |
| 24_h | Needle | WN | MN | 1.4936 | 0.1874 | 2.7999 | 0.0302 | * |
| 24_h | SF | WN | MN | 1.6467 | -1.4926 | 4.7860 | 0.2239 | ns |
| 72_h | Ctrl | WN | MN | 0.4045 | -2.1397 | 2.9487 | 0.7043 | ns |
| 72_h | Needle | WN | MN | -0.9538 | -3.6657 | 1.7581 | 0.4279 | ns |
| 72_h | SF | WN | MN | -1.3556 | -2.9135 | 0.2023 | 0.0795 | + |
| Predictor: Route | | | | | | | | |
| 24_h | WN | Ctrl | Needle | -1.3581 | -2.8960 | 0.1798 | 0.0309 | * |
| 24_h | WN | Ctrl | SF | -4.9856 | -6.3932 | -3.5781 | 0.0001 | **** |
| 24_h | WN | Needle | SF | -3.6276 | -4.8538 | -2.4014 | 0.0001 | **** |
| 24_h | MN | Ctrl | Needle | -0.2689 | -3.6094 | 3.0716 | 0.8036 | ns |

| | | | | | | | | |
|------|----|--------|--------|---------|---------|--------|--------|----|
| 24_h | MN | Ctrl | SF | -3.7434 | -8.1407 | 0.6539 | 0.0534 | + |
| 24_h | MN | Needle | SF | -3.4745 | -7.5673 | 0.6183 | 0.0534 | + |
| 72_h | WN | Ctrl | Needle | -0.0042 | -2.1190 | 2.1106 | 0.9953 | ns |
| 72_h | WN | Ctrl | SF | -0.7923 | -2.5263 | 0.9418 | 0.4595 | ns |
| 72_h | WN | Needle | SF | -0.7881 | -2.9489 | 1.3727 | 0.4595 | ns |
| 72_h | MN | Ctrl | Needle | -1.3625 | -5.3495 | 2.6245 | 0.3412 | ns |
| 72_h | MN | Ctrl | SF | -2.5524 | -5.9102 | 0.8053 | 0.1555 | ns |
| 72_h | MN | Needle | SF | -1.1900 | -4.6858 | 2.3059 | 0.3412 | ns |

Monocytes

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the Monocytes dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Monocytes counts per pooled ear single-cell suspension (Appendix table 15). The test output showed that only the “Route” predictor was a statistically significant factor. No statistically significant interaction between any predictors was observed.

Appendix Table 15
Monocytes: Robust three-way ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|---------|---------|------|
| Diet | 0.4677 | 0.5100 | ns |
| Route | 47.2342 | 0.0001 | **** |
| Time_point | 3.5637 | 0.0760 | + |
| Diet:Route | 6.4411 | 0.0830 | + |
| Diet:Time_point | 2.4362 | 0.1370 | ns |
| Route:Time_point | 3.9590 | 0.1950 | ns |
| Diet:Route:Time_point | 2.2057 | 0.3830 | ns |

We looked for main effects by splitting the data by each predictor separately and analyzed the respective remaining two predictor by a Robust two-way ANOVA. The results showed that, after adjustment of p-values for multiple tests, only the “Route” predictor produced statistically significant p-values, regardless of whether the data was split by “Diet” or “Time-point” (Appendix table 16). This suggested that the only predictor of impact on cell counts was the route of infection. No statistically significant interactions were observed at this level.

Appendix Table 16
Monocytes: Robust two-way ANOVA

| Grouper | Predictor | value | p.value | Sig. | p.value.adj | sig. |
|------------------|-----------|-------|---------|------|-------------|------|
| Grouped by Route | | | | | | |

| | | | | | | |
|--------|-----------------|--------|-------|----|--------|----|
| Ctrl | Diet | 5.2338 | 0.037 | * | 0.1110 | ns |
| Ctrl | Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Ctrl | Diet:Time_point | 0.0000 | 0.999 | ns | 0.9990 | ns |
| Needle | Diet | 0.0138 | 0.909 | ns | 0.9990 | ns |
| Needle | Time_point | 6.2017 | 0.027 | * | 0.0945 | + |
| Needle | Diet:Time_point | 4.8351 | 0.047 | * | 0.1234 | ns |
| SF | Diet | 0.7354 | 0.413 | ns | 0.5964 | ns |
| SF | Time_point | 0.7811 | 0.399 | ns | 0.5964 | ns |
| SF | Diet:Time_point | 0.8265 | 0.387 | ns | 0.5964 | ns |

Grouped by Diet

| | | | | | | |
|----|------------------|---------|-------|-----|--------|----|
| WN | Route | 36.6533 | 0.001 | *** | 0.0070 | ** |
| WN | Time_point | 0.0245 | 0.878 | ns | 0.9990 | ns |
| WN | Route:Time_point | 0.0477 | 0.978 | ns | 0.9990 | ns |
| MN | Route | 32.3071 | 0.001 | *** | 0.0070 | ** |
| MN | Time_point | 5.9000 | 0.025 | * | 0.0945 | + |
| MN | Route:Time_point | 5.5106 | 0.107 | ns | 0.2043 | ns |

Grouped by Time_point

| | | | | | | |
|------|------------|---------|-------|-----|--------|----|
| 24_h | Diet | 3.9779 | 0.065 | + | 0.1517 | ns |
| 24_h | Route | 21.9364 | 0.002 | ** | 0.0105 | * |
| 24_h | Diet:Route | 1.6608 | 0.478 | ns | 0.6274 | ns |
| 72_h | Diet | 0.6652 | 0.426 | ns | 0.5964 | ns |
| 72_h | Route | 46.8385 | 0.001 | *** | 0.0070 | ** |
| 72_h | Diet:Route | 6.2872 | 0.078 | + | 0.1638 | ns |

For the analysis of the simple simple main effect for each respective predictor, we performed Robust one-way ANOVA with individual predictors of the data split by the other two predictors. The output showed that after the adjustment of p-values for multiple tests, only the “Route” predictor produce statistically significant p-values in the malnourished group at 72 h post infection (Appendix table 17).

Appendix Table 17
Monocytes: Robust one-way ANOVA

| Predictor_1 | Predictor_2 | Effect | test | df1 | df2 | p.value | effsize | p.value.adj | sig. |
|-----------------------|-------------|------------|--------|-----|--------|---------|---------|-------------|------|
| Predictor: Time_point | | | | | | | | | |
| Ctrl | WN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Ctrl | MN | Time_point | 0.0000 | 1 | 8.0000 | 1.0000 | 0.0000 | 1.0000 | ns |
| Needle | WN | Time_point | 0.0512 | 1 | 6.2708 | 0.8281 | 0.1320 | 1.0000 | ns |
| Needle | MN | Time_point | 9.3895 | 1 | 5.7100 | 0.0236 | 0.9343 | 0.0942 | + |
| SF | WN | Time_point | 0.0010 | 1 | 7.9009 | 0.9756 | 0.0126 | 1.0000 | ns |

| | | | | | | | | | |
|------------------|--------|------------|---------|---|--------|--------|--------|--------|----|
| SF | MN | Time_point | 0.9577 | 1 | 6.3626 | 0.3635 | 0.5490 | 0.5287 | ns |
| Predictor: Diet | | | | | | | | | |
| 24_h | Ctrl | Diet | 2.6169 | 1 | 7.0286 | 0.1496 | 0.5569 | 0.2659 | ns |
| 24_h | Needle | Diet | 6.5185 | 1 | 7.9850 | 0.0341 | 0.8413 | 0.1090 | ns |
| 24_h | SF | Diet | 0.0009 | 1 | 4.8921 | 0.9767 | 0.0196 | 1.0000 | ns |
| 72_h | Ctrl | Diet | 2.6169 | 1 | 7.0286 | 0.1496 | 0.5569 | 0.2659 | ns |
| 72_h | Needle | Diet | 1.3637 | 1 | 7.6805 | 0.2779 | 0.5236 | 0.4446 | ns |
| 72_h | SF | Diet | 2.6348 | 1 | 6.9154 | 0.1491 | 0.6089 | 0.2659 | ns |
| Predictor: Route | | | | | | | | | |
| 24_h | WN | Route | 9.5871 | 2 | 7.8674 | 0.0078 | 0.8982 | 0.0622 | + |
| 24_h | MN | Route | 4.6108 | 2 | 7.4049 | 0.0501 | 0.7502 | 0.1335 | ns |
| 72_h | WN | Route | 7.7990 | 2 | 7.5548 | 0.0145 | 0.6443 | 0.0776 | + |
| 72_h | MN | Route | 14.2513 | 2 | 7.7234 | 0.0026 | 0.7890 | 0.0409 | * |

For the pairwise comparison, we applied a Linear contrast expression. The output showed that at 24 h post infection, the sand fly infection route was statistically significant from the control and needle inoculum in the well-nourished (WN) group (Appendix table 18). At 72 h, the sand fly infection route was statistically significant from the control in the malnourished group. Further, statistically significant difference were observed between well-nourished and malnourished mice inoculated by needle at 24 h post infection and infected by sand fly at 72 h post infection for the needle and sand fly inoculum, respectively.

Appendix Table 18
Monocytes: Pairwise comparison by Linear Contrast Expression

| Predictor_1 | Predictor_2 | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------------------|-------------|-------|---------|---------|----------|----------|---------|------|
| Predictor: Time_point | | | | | | | | |
| Ctrl | WN | 24_h | 72_h | 0.0000 | -0.0791 | 0.0791 | 1.0000 | ns |
| Ctrl | MN | 24_h | 72_h | 0.0000 | -0.1169 | 0.1169 | 1.0000 | ns |
| Needle | WN | 24_h | 72_h | -0.0114 | -0.1337 | 0.1108 | 0.8281 | ns |
| Needle | MN | 24_h | 72_h | -0.1838 | -0.3324 | -0.0352 | 0.0236 | * |
| SF | WN | 24_h | 72_h | 0.0009 | -0.0646 | 0.0664 | 0.9756 | ns |
| SF | MN | 24_h | 72_h | -0.0633 | -0.2195 | 0.0928 | 0.3635 | ns |
| Predictor: Diet | | | | | | | | |
| 24_h | Ctrl | WN | MN | 0.0700 | -0.0322 | 0.1722 | 0.1496 | ns |
| 24_h | Needle | WN | MN | 0.0908 | 0.0088 | 0.1728 | 0.0341 | * |
| 24_h | SF | WN | MN | 0.0018 | -0.1515 | 0.1551 | 0.9767 | ns |
| 72_h | Ctrl | WN | MN | 0.0700 | -0.0322 | 0.1722 | 0.1496 | ns |
| 72_h | Needle | WN | MN | -0.0816 | -0.2439 | 0.0807 | 0.2779 | ns |

| | | | | | | | | |
|------------------|----|--------|--------|---------|---------|---------|--------|----|
| 72_h | SF | WN | MN | -0.0624 | -0.1535 | 0.0287 | 0.1491 | ns |
| Predictor: Route | | | | | | | | |
| 24_h | WN | Ctrl | Needle | -0.0393 | -0.1416 | 0.0629 | 0.2879 | ns |
| 24_h | WN | Ctrl | SF | -0.1324 | -0.2246 | -0.0401 | 0.0089 | ** |
| 24_h | WN | Needle | SF | -0.0930 | -0.1863 | 0.0002 | 0.0276 | * |
| 24_h | MN | Ctrl | Needle | -0.0185 | -0.1522 | 0.1152 | 0.6868 | ns |
| 24_h | MN | Ctrl | SF | -0.2005 | -0.4060 | 0.0049 | 0.0418 | * |
| 24_h | MN | Needle | SF | -0.1820 | -0.3834 | 0.0194 | 0.0418 | * |
| 72_h | WN | Ctrl | Needle | -0.0507 | -0.2096 | 0.1081 | 0.3508 | ns |
| 72_h | WN | Ctrl | SF | -0.1315 | -0.2271 | -0.0359 | 0.0109 | * |
| 72_h | WN | Needle | SF | -0.0807 | -0.2388 | 0.0774 | 0.2280 | ns |
| 72_h | MN | Ctrl | Needle | -0.2023 | -0.4017 | -0.0030 | 0.0258 | * |
| 72_h | MN | Ctrl | SF | -0.2638 | -0.4067 | -0.1210 | 0.0018 | ** |
| 72_h | MN | Needle | SF | -0.0615 | -0.2581 | 0.1351 | 0.3639 | ns |

Statistical power

Considering the small group $N=5$ and the low occurrence of statistical significant outcomes, it stood to reason that the study design was statistically underpowered by necessity to keep animal numbers and costs manageable. Thus, we performed a retrospective power analysis on the data by cell-type group to explore this.

Effect size estimation based on partial η^2

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 19 to 21 show the respective effect sizes. Note that upper ends of the confidence intervals were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial η^2 values from the effect size calculation were then used for the retrospective power calculations.

Appendix Table 19
Myeloid cells: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size | Effect Size |
|------------------|--------------|--------|---------|-------------|-------------|
| Diet | 0.0073 | 0.0000 | 1 | very small | very small |
| Route | 0.5033 | 0.3277 | 1 | large | large |
| Time_point | 0.0017 | 0.0000 | 1 | very small | very small |
| Diet:Route | 0.0398 | 0.0000 | 1 | small | small |
| Diet:Time_point | 0.1270 | 0.0174 | 1 | small | medium |
| Route:Time_point | 0.0125 | 0.0000 | 1 | very small | small |

| | | | | | |
|-----------------------|--------|--------|---|-------|--------|
| Diet:Route:Time_point | 0.0689 | 0.0000 | 1 | small | medium |
|-----------------------|--------|--------|---|-------|--------|

Appendix Table 20
Neutrophils: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size | Effect Size |
|-----------------------|--------------|--------|---------|-------------|-------------|
| Diet | 0.0041 | 0.0000 | 1 | very small | very small |
| Route | 0.5220 | 0.3496 | 1 | large | large |
| Time_point | 0.1716 | 0.0398 | 1 | medium | large |
| Diet:Route | 0.0016 | 0.0000 | 1 | very small | very small |
| Diet:Time_point | 0.1087 | 0.0101 | 1 | small | medium |
| Route:Time_point | 0.2980 | 0.1175 | 1 | large | large |
| Diet:Route:Time_point | 0.0645 | 0.0000 | 1 | small | medium |

Appendix Table 21
Monocytes: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size | Effect Size |
|-----------------------|--------------|--------|---------|-------------|-------------|
| Diet | 0.0204 | 0.0000 | 1 | small | small |
| Route | 0.4711 | 0.2909 | 1 | large | large |
| Time_point | 0.0894 | 0.0035 | 1 | small | medium |
| Diet:Route | 0.0775 | 0.0000 | 1 | small | medium |
| Diet:Time_point | 0.0668 | 0.0000 | 1 | small | medium |
| Route:Time_point | 0.0733 | 0.0000 | 1 | small | medium |
| Diet:Route:Time_point | 0.0476 | 0.0000 | 1 | small | small |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power. For small mean differences within data with a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. For Myeloid_cells, only the predictor(s) “Route” and in interaction(s) “Diet:Time_point” resulted in optimal sample sizes that were within the total sample size of $N=60$, 60, 60 (Appendix table 22). The large proposed sample sizes for “Diet” and “Time_point” on their own suggested little statistically significant difference between groups by individual predictor. Thus, the most meaningful predictor for Myeloid_cells counts was the route of infection; by needle, sand fly, or no-infection (control).

Appendix Table 22
Myeloid Cells: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|--------|-------|---------|-----|-----|-----|
|--------|-------|---------|-----|-----|-----|

| | | | | | |
|-----------------------|-----|------|--------|---|----------|
| Diet | | | | | |
| Diet | 0.8 | 1067 | 7.863 | 1 | 1064.116 |
| Route | | | | | |
| Route | 0.8 | 14 | 13.197 | 2 | 10.022 |
| Time_point | | | | | |
| Time_point | 0.8 | 4599 | 7.852 | 1 | 4596.543 |
| Diet:Route | | | | | |
| Diet | 0.8 | 192 | 7.931 | 1 | 185.328 |
| Route | 0.8 | 236 | 9.762 | 2 | 229.489 |
| Diet:Route | 0.8 | 236 | 9.762 | 2 | 229.489 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 57 | 8.149 | 1 | 52.006 |
| Time_point | 0.8 | 57 | 8.149 | 1 | 52.006 |
| Diet:Time_point | 0.8 | 57 | 8.149 | 1 | 52.006 |
| Route:Time_point | | | | | |
| Route | 0.8 | 762 | 9.673 | 2 | 755.924 |
| Time_point | 0.8 | 621 | 7.873 | 1 | 614.181 |
| Route:Time_point | 0.8 | 762 | 9.673 | 2 | 755.924 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 109 | 8.008 | 1 | 96.268 |
| Route | 0.8 | 134 | 9.876 | 2 | 121.519 |
| Time_point | 0.8 | 109 | 8.008 | 1 | 96.268 |
| Diet:Route | 0.8 | 134 | 9.876 | 2 | 121.519 |
| Diet:Time_point | 0.8 | 109 | 8.008 | 1 | 96.268 |
| Route:Time_point | 0.8 | 134 | 9.876 | 2 | 121.519 |
| Diet:Route:Time_point | 0.8 | 134 | 9.876 | 2 | 121.519 |

Similar observation were made for Neutrophils counts, but here “Time_point” was a more meaningful predictor. Alongside “Route”, proposed sample sizes were well within the total sample size of $N=60$, 60, 60 (Appendix Table 23).

Appendix Table 23
Neutrophils: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|--------|-------|---------|-----|-----|-----|
|--------|-------|---------|-----|-----|-----|

| | | | | | |
|-----------------------|-----|------|--------|---|----------|
| Diet | | | | | |
| Diet | 0.8 | 1929 | 7.857 | 1 | 1926.454 |
| Route | | | | | |
| Route | 0.8 | 13 | 13.507 | 2 | 9.366 |
| Time_point | | | | | |
| Time_point | 0.8 | 40 | 8.266 | 1 | 37.898 |
| Diet:Route | | | | | |
| Diet | 0.8 | 4780 | 7.852 | 1 | 4773.506 |
| Route | 0.8 | 5868 | 9.640 | 2 | 5861.611 |
| Diet:Route | 0.8 | 5868 | 9.640 | 2 | 5861.611 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 67 | 8.098 | 1 | 62.403 |
| Time_point | 0.8 | 67 | 8.098 | 1 | 62.403 |
| Diet:Time_point | 0.8 | 67 | 8.098 | 1 | 62.403 |
| Route:Time_point | | | | | |
| Route | 0.8 | 27 | 11.202 | 2 | 20.386 |
| Time_point | 0.8 | 22 | 8.975 | 1 | 15.140 |
| Route:Time_point | 0.8 | 27 | 11.202 | 2 | 20.386 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 117 | 7.996 | 1 | 104.054 |
| Route | 0.8 | 144 | 9.858 | 2 | 131.079 |
| Time_point | 0.8 | 117 | 7.996 | 1 | 104.054 |
| Diet:Route | 0.8 | 144 | 9.858 | 2 | 131.079 |
| Diet:Time_point | 0.8 | 117 | 7.996 | 1 | 104.054 |
| Route:Time_point | 0.8 | 144 | 9.858 | 2 | 131.079 |
| Diet:Route:Time_point | 0.8 | 144 | 9.858 | 2 | 131.079 |

For Monocytes counts, “Diet” became a more meaningful predictor, although it would still have required a 6.3 times larger total sample size than was available for the study (Appendix Table 24). “Time_point” was about as meaningful as it had been for the Myeloid_cells. Thus, only “Route” was a meaningful predictor that was on its own within proposed sample sizes ranges.

Appendix Table 24
Monocytes: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|--------|-------|---------|-----|-----|-----|
|--------|-------|---------|-----|-----|-----|

| | | | | | |
|-----------------------|-----|-----|--------|---|---------|
| Diet | | | | | |
| Diet | 0.8 | 380 | 7.889 | 1 | 377.085 |
| Route | | | | | |
| Route | 0.8 | 15 | 12.722 | 2 | 11.281 |
| Time_point | | | | | |
| Time_point | 0.8 | 82 | 8.042 | 1 | 79.941 |
| Diet:Route | | | | | |
| Diet | 0.8 | 96 | 8.021 | 1 | 89.461 |
| Route | 0.8 | 118 | 9.898 | 2 | 111.798 |
| Diet:Route | 0.8 | 118 | 9.898 | 2 | 111.798 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 112 | 7.991 | 1 | 107.66 |
| Time_point | 0.8 | 112 | 7.991 | 1 | 107.66 |
| Diet:Time_point | 0.8 | 112 | 7.991 | 1 | 107.66 |
| Route:Time_point | | | | | |
| Route | 0.8 | 125 | 9.882 | 2 | 118.891 |
| Time_point | 0.8 | 102 | 8.010 | 1 | 95.238 |
| Route:Time_point | 0.8 | 125 | 9.882 | 2 | 118.891 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 159 | 7.953 | 1 | 146.953 |
| Route | 0.8 | 196 | 9.794 | 2 | 183.745 |
| Time_point | 0.8 | 159 | 7.953 | 1 | 146.953 |
| Diet:Route | 0.8 | 196 | 9.794 | 2 | 183.745 |
| Diet:Time_point | 0.8 | 159 | 7.953 | 1 | 146.953 |
| Route:Time_point | 0.8 | 196 | 9.794 | 2 | 183.745 |
| Diet:Route:Time_point | 0.8 | 196 | 9.794 | 2 | 183.745 |

Retrospective calculation of statistical power in our data analysis

The observations from the retrospective minimum sample size calculation were reflected in the power calculation for our data. For Myeloid_cells counts, only “Route” and the interaction of “Diet:Time_point” had sufficient statistical power to have a change to find statistically meaning differences in the data (Appendix Table 25).

Appendix Table 25
Myeloid Cells: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|-----|
| Diet | | | | | |
| Diet | 0.1 | 60 | 0.443 | 1 | 58 |
| Route | | | | | |
| Route | 1 | 60 | 60.805 | 2 | 57 |
| Time_point | | | | | |
| Time_point | 0.061 | 60 | 0.102 | 1 | 58 |
| Diet:Route | | | | | |
| Diet | 0.341 | 60 | 2.487 | 1 | 54 |
| Route | 0.259 | 60 | 2.487 | 2 | 54 |
| Diet:Route | 0.259 | 60 | 2.487 | 2 | 54 |
| Diet:Time_point | | | | | |
| Diet | 0.827 | 60 | 8.73 | 1 | 56 |
| Time_point | 0.827 | 60 | 8.73 | 1 | 56 |
| Diet:Time_point | 0.827 | 60 | 8.73 | 1 | 56 |
| Route:Time_point | | | | | |
| Route | 0.108 | 60 | 0.762 | 2 | 54 |
| Time_point | 0.138 | 60 | 0.762 | 1 | 54 |
| Route:Time_point | 0.108 | 60 | 0.762 | 2 | 54 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.542 | 60 | 4.438 | 1 | 48 |
| Route | 0.431 | 60 | 4.438 | 2 | 48 |
| Time_point | 0.542 | 60 | 4.438 | 1 | 48 |
| Diet:Route | 0.431 | 60 | 4.438 | 2 | 48 |
| Diet:Time_point | 0.542 | 60 | 4.438 | 1 | 48 |
| Route:Time_point | 0.431 | 60 | 4.438 | 2 | 48 |
| Diet:Route:Time_point | 0.431 | 60 | 4.438 | 2 | 48 |

For Neutrophils counts, “Route” and “Time_points as much as their interaction had >80% statistical power, while the interaction”Diet:Time_point” was close to 80% statistical power (Appendix Table 26).

Appendix Table 26
Neutrophils: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|-----|
| Diet | | | | | |
| Diet | 0.078 | 60 | 0.244 | 1 | 58 |
| Route | | | | | |
| Route | 1 | 60 | 65.534 | 2 | 57 |
| Time_point | | | | | |
| Time_point | 0.934 | 60 | 12.431 | 1 | 58 |
| Diet:Route | | | | | |
| Diet | 0.061 | 60 | 0.099 | 1 | 54 |
| Route | 0.057 | 60 | 0.099 | 2 | 54 |
| Diet:Route | 0.057 | 60 | 0.099 | 2 | 54 |
| Diet:Time_point | | | | | |
| Diet | 0.758 | 60 | 7.317 | 1 | 56 |
| Time_point | 0.758 | 60 | 7.317 | 1 | 56 |
| Diet:Time_point | 0.758 | 60 | 7.317 | 1 | 56 |
| Route:Time_point | | | | | |
| Route | 0.995 | 60 | 25.473 | 2 | 54 |
| Time_point | 0.999 | 60 | 25.473 | 1 | 54 |
| Route:Time_point | 0.995 | 60 | 25.473 | 2 | 54 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.513 | 60 | 4.134 | 1 | 48 |
| Route | 0.405 | 60 | 4.134 | 2 | 48 |
| Time_point | 0.513 | 60 | 4.134 | 1 | 48 |
| Diet:Route | 0.405 | 60 | 4.134 | 2 | 48 |
| Diet:Time_point | 0.513 | 60 | 4.134 | 1 | 48 |
| Route:Time_point | 0.405 | 60 | 4.134 | 2 | 48 |
| Diet:Route:Time_point | 0.405 | 60 | 4.134 | 2 | 48 |

Similar to Myeloid_cells counts, for Monocytes counts, “Route” was the only predictor that produce >80% statistical power on its own, but not in any interaction with “Diet” and/or “Time_point” (Appendix Table 27).

Appendix Table 27
Monocytes: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|-----|
| Diet | | | | | |
| Diet | 0.196 | 60 | 1.249 | 1 | 58 |
| Route | | | | | |
| Route | 1 | 60 | 53.449 | 2 | 57 |
| Time_point | | | | | |
| Time_point | 0.665 | 60 | 5.888 | 1 | 58 |
| Diet:Route | | | | | |
| Diet | 0.597 | 60 | 5.041 | 1 | 54 |
| Route | 0.484 | 60 | 5.041 | 2 | 54 |
| Diet:Route | 0.484 | 60 | 5.041 | 2 | 54 |
| Diet:Time_point | | | | | |
| Diet | 0.531 | 60 | 4.294 | 1 | 56 |
| Time_point | 0.531 | 60 | 4.294 | 1 | 56 |
| Diet:Time_point | 0.531 | 60 | 4.294 | 1 | 56 |
| Route:Time_point | | | | | |
| Route | 0.460 | 60 | 4.747 | 2 | 54 |
| Time_point | 0.571 | 60 | 4.747 | 1 | 54 |
| Route:Time_point | 0.460 | 60 | 4.747 | 2 | 54 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.397 | 60 | 3.002 | 1 | 48 |
| Route | 0.304 | 60 | 3.002 | 2 | 48 |
| Time_point | 0.397 | 60 | 3.002 | 1 | 48 |
| Diet:Route | 0.304 | 60 | 3.002 | 2 | 48 |
| Diet:Time_point | 0.397 | 60 | 3.002 | 1 | 48 |
| Route:Time_point | 0.304 | 60 | 3.002 | 2 | 48 |
| Diet:Route:Time_point | 0.304 | 60 | 3.002 | 2 | 48 |

Conclusion

In conclusion, it can be said that the infection route (needle, sand fly, or uninfected) was the primary difference maker with respect to observed Myeloid_cells, Neutrophils and Monocytes counts in pooled mouse ear single-cell suspensions. It is of note that the nutritional status of the individuals did not have a

detectable impact on the observed cell counts in the site of infection.

Panel e and f

Data analysis

Figure 1 e and f presented the same samples as shown in figure 1b and c with the added difference that only IL-1 β ⁺ cells were counted. Again, the outcome variable of total IL-1 β ⁺ Myeloid_cells counts and separately, IL-1 β ⁺ Neutrophils and Monocytes counts from $N=40$, 40, 40 pools of single cell suspensions, respectively, prepared from BALB/c mouse ears collected 24 h and 72 h post infection with *Leishmania donovani* by “needle” inoculation or infective sand flies (SF) bites. Please, refer to the methods section of the publication for more details on sample preparation. Note that different mice were sampled at 24 h and 72 h post infection, which meant that this dataset satisfied the independence of data points and therefore, did not represent a repeatedly measured dataset. Thus, this dataset contained three between-subject factors, “Diet” (well-nourished [WN] or malnourished [MN]), “Route” (uninfected control [Ctrl], needle inoculation [Needle], infective sand fly bites [SF]) and “Time_point” (collection at 24 h or 72 h). Based on this information, a three-way analysis was indicated.

Thus, we assessed the data for compliance with assumptions for a three-way ANOVA:

- Data normality
- Homogeneity of variance
- No significant outliers

Initial assumption assessment indicated that data transformation was required to meet assumptions for a three-way ANOVA only for the Neutrophils counts. Thus, we settled for a Box-Cox power transformation. Conversely, Monocytes did not need to be transformed to apply a Robust three-way ANOVA, while Neutrophils were analyzed by Simple linear regression post Box-Cox power transformation. The data transformation resulted in different data distributions and variances than appear in the main figure in the publication.

Assumption analyses

Data normality

The assessment of the transformed data distribution for each group was conducted by Shpiro-Wilks test and QQ-plot for counts of Myeloid_cells, Neutrophils and Monocytes separately. Note that all groups of all datasets consisted of $N=5$ pools of mouse ear single-cell suspensions, which made it difficult to assess data distribution reliably by Shapiro-Wilks test.

Myeloid_cells

In spite of assessment limitations due to small group sizes, we concluded based on Shapiro-Wilks test (Appendix table 28) and QQ-plots (Fig.1e-f-1) that all groups of the dataset were likely to follow a normal

distribution with the potential exception of well-nourished, sand fly inoculated mice at 24 h post infection.

Appendix Table 28
Myeloid cells: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Needle | 24_h | Counts | 0.9773 | 0.9198 | ns |
| WN | Needle | 72_h | Counts | 0.9486 | 0.7275 | ns |
| MN | Needle | 24_h | Counts | 0.8686 | 0.2608 | ns |
| MN | Needle | 72_h | Counts | 0.9146 | 0.4960 | ns |
| WN | SF | 24_h | Counts | 0.7364 | 0.0222 | sig. |
| WN | SF | 72_h | Counts | 0.8934 | 0.3742 | ns |
| MN | SF | 24_h | Counts | 0.8915 | 0.3649 | ns |
| MN | SF | 72_h | Counts | 0.9664 | 0.8520 | ns |

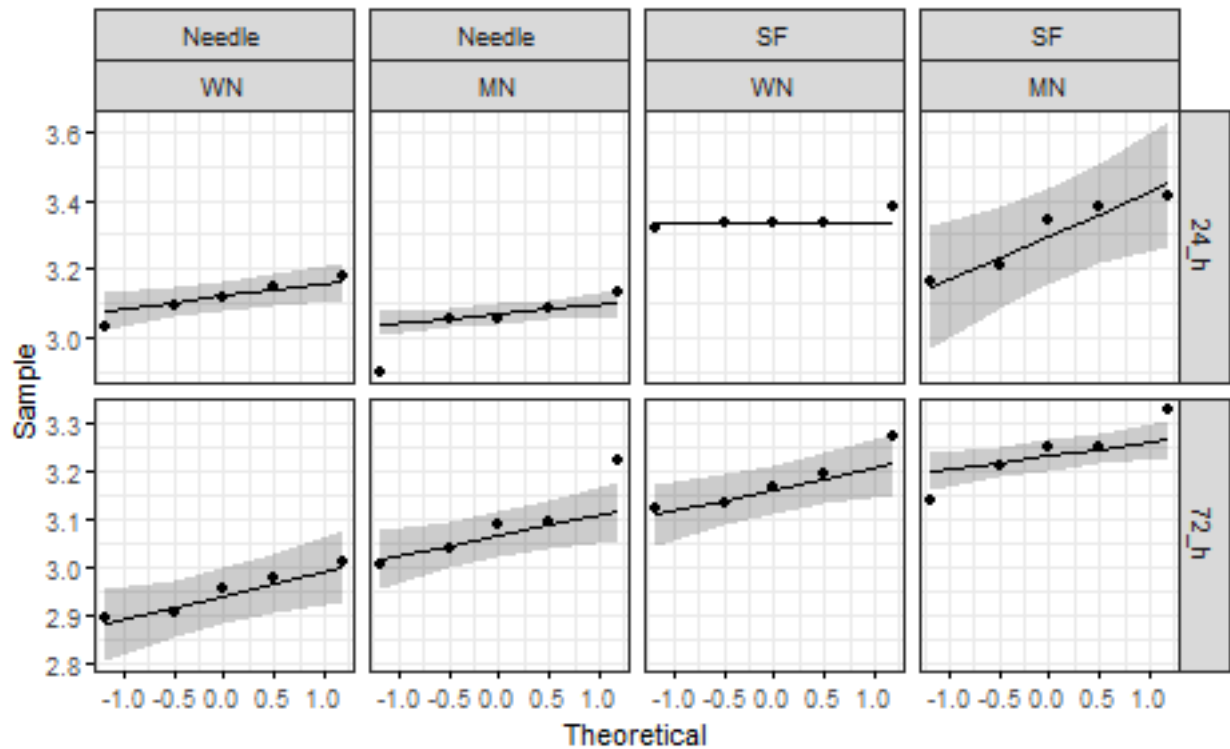


Fig.1e-f-1: QQ-plots of myeloid cell counts split into groups by predictor variables

Neutrophils

In spite of assessment limitations due to small group sizes, We concluded based on Shapiro-Wilks test (Appendix table 29) and QQ-plots (Fig.1e-f-2) that all groups of the dataset were likely to follow a normal distribution.

Appendix Table 29
Neutrophils: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Needle | 24_h | Counts | 0.9664 | 0.8514 | ns |
| WN | Needle | 72_h | Counts | 0.9196 | 0.5271 | ns |
| MN | Needle | 24_h | Counts | 0.9650 | 0.8420 | ns |
| MN | Needle | 72_h | Counts | 0.8507 | 0.1967 | ns |
| WN | SF | 24_h | Counts | 0.9704 | 0.8776 | ns |
| WN | SF | 72_h | Counts | 0.9251 | 0.5632 | ns |
| MN | SF | 24_h | Counts | 0.9265 | 0.5725 | ns |
| MN | SF | 72_h | Counts | 0.9361 | 0.6383 | ns |

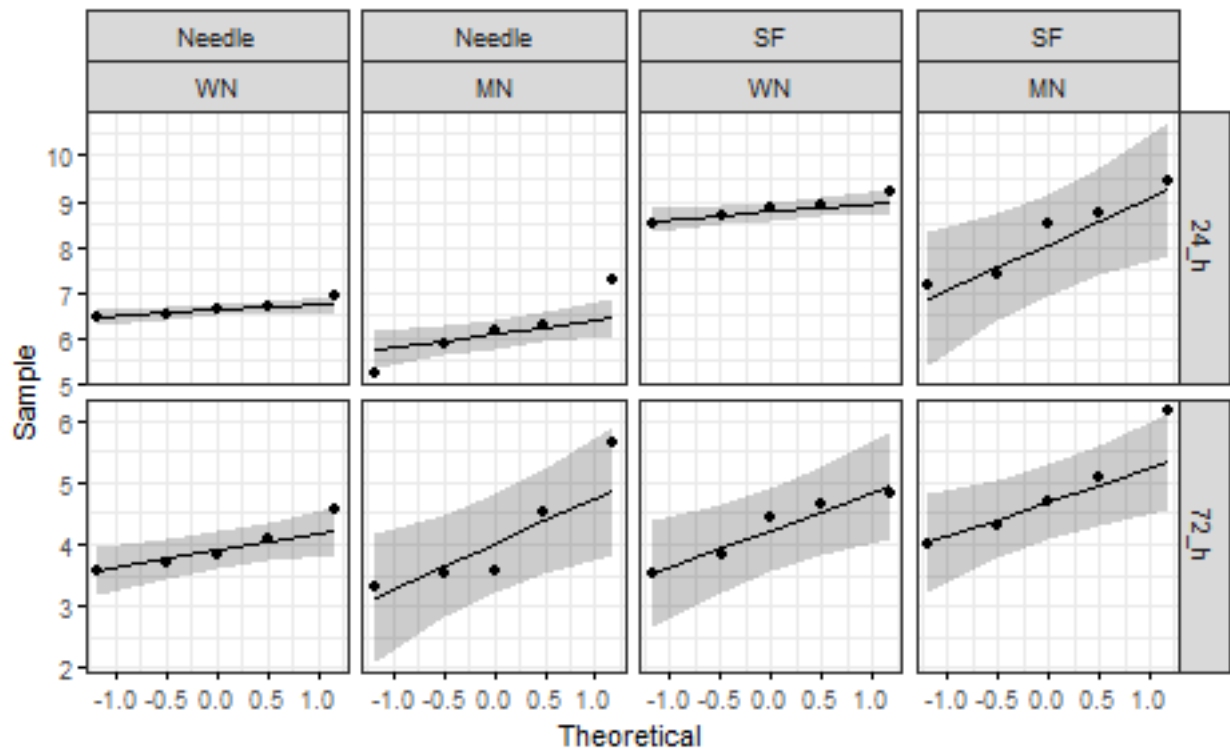


Fig.1e-f-2: QQ-plots of neutrophil counts split into groups by predictor variables

Monocytes

In spite of assessment limitations due to small group sizes, we concluded based on Shapiro-Wilks test (Appendix table 30) and QQ-plots (Fig.1e-f-3) that all groups of the dataset were likely to follow a normal distribution.

Appendix Table 30
Monocytes: Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|------|--------|------------|----------|-----------|--------|---------|
| WN | Needle | 24_h | Counts | 0.9542 | 0.7674 | ns |
| WN | Needle | 72_h | Counts | 0.8933 | 0.3740 | ns |
| MN | Needle | 24_h | Counts | 0.9561 | 0.7806 | ns |
| MN | Needle | 72_h | Counts | 0.8828 | 0.3221 | ns |
| WN | SF | 24_h | Counts | 0.8563 | 0.2151 | ns |
| WN | SF | 72_h | Counts | 0.9219 | 0.5422 | ns |
| MN | SF | 24_h | Counts | 0.9430 | 0.6874 | ns |
| MN | SF | 72_h | Counts | 0.8849 | 0.3319 | ns |

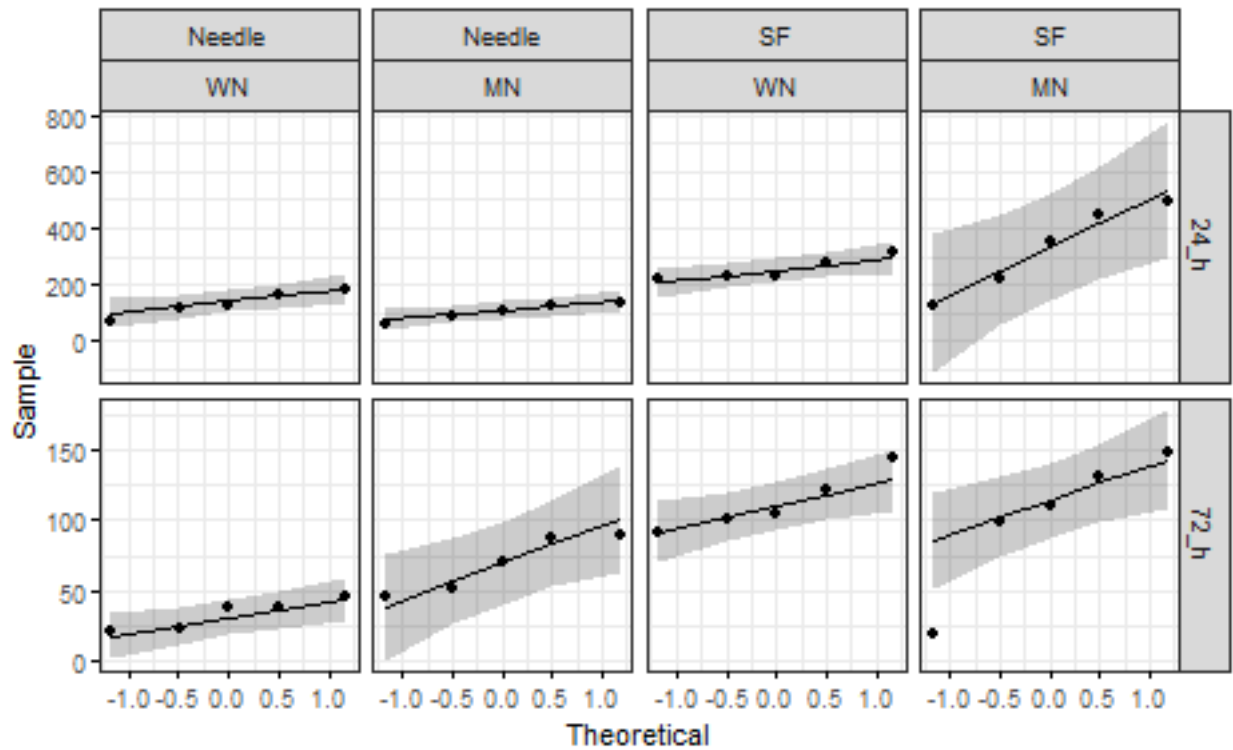


Fig.1e-f-3: QQ-plots of monocyte counts split into groups by predictor variables

Homogeneity of variance The assessment of homogeneity of variance was also conducted for myeloid cell, neutrophil and monocyte counts separately. We employed the Levene's test on each dataset. The p-value for the test suggested that the assumption of homogeneity of variance held for Myeloid_cells ($p=0.5389$), held for Neutrophils ($p=0.401$), and was rejected for Monocytes ($p=0.0011$).

Outliers It can be difficult to determine outliers in small datasets reliably as the analysis is dependent on the interquartile range of the data per group. We attempted it anyway and found a potential 6 outliers in

the Myeloid_cells dataset (Appendix table 31), 2 outliers in the Neutrophils dataset (Appendix table 32), and 1 outliers in the Monocytes dataset (Appendix table 33), of which some were classified as extreme. However, larger datasets may have found these not be outliers, but just peripheral.

Appendix Table 31
Myeloid cells: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|--------|------------|------------|------------|
| MN | Needle | 24_h | TRUE | TRUE |
| MN | Needle | 72_h | TRUE | FALSE |
| WN | SF | 24_h | TRUE | TRUE |
| WN | SF | 24_h | TRUE | TRUE |
| MN | SF | 72_h | TRUE | FALSE |
| MN | SF | 72_h | TRUE | FALSE |

Appendix Table 32
Neutrophils: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|--------|------------|------------|------------|
| MN | Needle | 24_h | TRUE | FALSE |
| MN | Needle | 24_h | TRUE | FALSE |

Appendix Table 33
Monocytes: List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------|-------|------------|------------|------------|
| MN | SF | 72_h | TRUE | FALSE |

Three-way analysis

The appropriate three-way analysis was performed for Myeloid_cells, Neutrophils and Monocytes separately.

Myeloid_cells

Based on the assumption tests, we decided to apply a Simple linear regression to the Myeloid_cells dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Myeloid_cells counts per pooled ear single-cell suspension (Appendix table 34). The appropriateness of the Simple linear regression was confirmed by checking the model residuals for normal distribution (Shapiro-Wilks test: 0.771; Fig.1e-f-4).

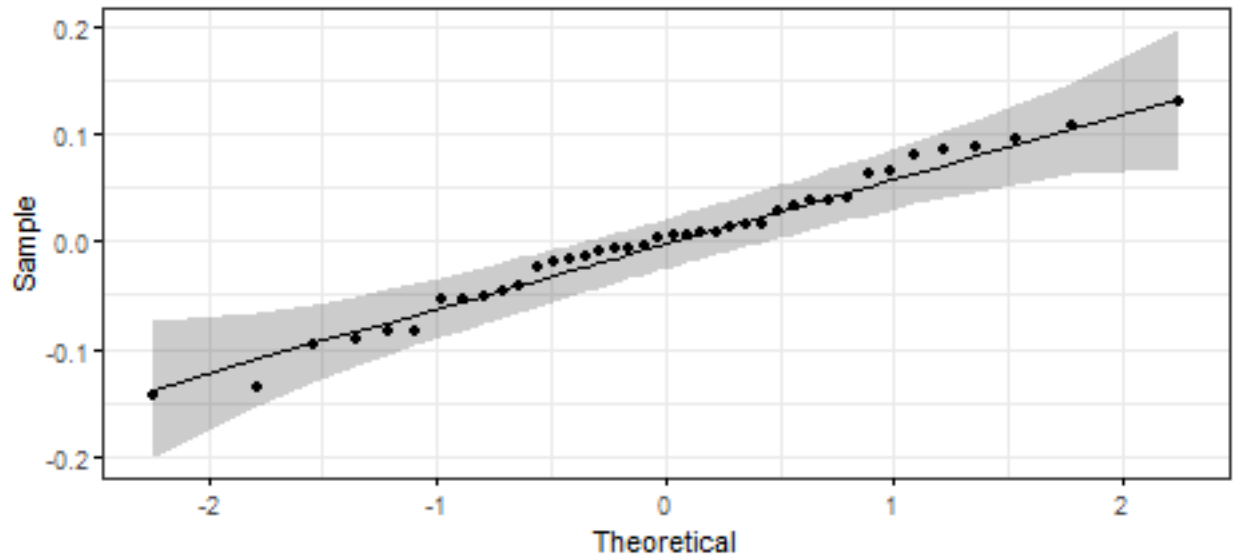


Fig.1e-f-4: QQ-plots of monocyte counts split into groups by predictor variables

The test output of the Simple linear regression showed that only the “Route” and “Time_point” were statistically significant predictors , while the interaction between Time_point and Diet was statistically significant, too. The importance of the “Time_point” predictor was a significant difference for the IL-1 β ⁺ Myeloid_cells counts, compared to observations in figure 1b & c, when IL-1 β ⁺ was not considered.

Appendix Table 34
Myeloid cells: Simple linear regression

| Predictors | Df | Sum Sq | Mean Sq | F value | Pr(>F) | sig. |
|-----------------------|----|--------|---------|---------|---------|------|
| Diet | 1 | 0.0056 | 0.0056 | 1.1286 | 0.2960 | ns |
| Route | 1 | 0.4567 | 0.4567 | 91.9654 | <0.0001 | **** |
| Time_point | 1 | 0.0750 | 0.0750 | 15.1095 | 0.0005 | *** |
| Diet:Route | 1 | 0.0016 | 0.0016 | 0.3309 | 0.5692 | ns |
| Diet:Time_point | 1 | 0.0585 | 0.0585 | 11.7781 | 0.0017 | ** |
| Route:Time_point | 1 | 0.0069 | 0.0069 | 1.3874 | 0.2475 | ns |
| Diet:Route:Time_point | 1 | 0.0080 | 0.0080 | 1.6065 | 0.2141 | ns |

As we performed a Simple linear regression, we were not able to analyze main and simple effects of predictors, but moved immediately to perform a pairwise comparison. We applied a Estimated marginal means analysis and the output showed that at 24 h post infection, the sand fly infection route was statistically significant from the control and needle inoculum in the well-nourished (WN) group (Appendix table 37). At 72 h, the sand fly infection route was statistically significant from the control in the malnourished group. Further, statistically significant difference were observed between well-nourished and malnourished mice inoculated by needle at 24 h post infection and infected by sand fly at 72 h post infection.

Appendix Table 37
Myeloid cells: Pairwise comparison by Estimated marginal means analysis

| Pairing | estimate | SE | df | t.ratio | p.value | sig. |
|---------------------------------|----------|--------|----|---------|---------|------|
| WN Needle 24_h - MN Needle 24_h | 0.0682 | 0.0446 | 32 | 1.5309 | 0.9831 | ns |
| WN Needle 24_h - WN SF 24_h | -0.2245 | 0.0446 | 32 | -5.0377 | 0.0005 | *** |
| WN Needle 24_h - WN Needle 72_h | 0.1651 | 0.0446 | 32 | 3.7043 | 0.0221 | * |
| MN Needle 24_h - MN SF 24_h | -0.2554 | 0.0446 | 32 | -5.7300 | 0.0001 | **** |
| MN Needle 24_h - MN Needle 72_h | -0.0443 | 0.0446 | 32 | -0.9951 | 1.0000 | ns |
| WN SF 24_h - MN SF 24_h | 0.0374 | 0.0446 | 32 | 0.8386 | 1.0000 | ns |
| WN SF 24_h - WN SF 72_h | 0.1611 | 0.0446 | 32 | 3.6147 | 0.0282 | * |
| MN SF 24_h - MN SF 72_h | 0.0646 | 0.0446 | 32 | 1.4503 | 0.9915 | ns |
| WN Needle 72_h - MN Needle 72_h | -0.1412 | 0.0446 | 32 | -3.1685 | 0.0900 | + |
| WN Needle 72_h - WN SF 72_h | -0.2285 | 0.0446 | 32 | -5.1274 | 0.0004 | *** |
| MN Needle 72_h - MN SF 72_h | -0.1464 | 0.0446 | 32 | -3.2846 | 0.0671 | + |
| WN SF 72_h - MN SF 72_h | -0.0591 | 0.0446 | 32 | -1.3258 | 0.9976 | ns |

Neutrophils

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the Neutrophils dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Neutrophils counts per pooled ear single-cell suspension (Appendix table 38). As for Myeloid_cells counts, the test output showed that “Route” and “Time_point” were both statistically significant predictors, while here, their interaction was also statistically meaningful rather than “Diet: and”Time_point”.

Appendix Table 38
Neutrophils: Robust three-way ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|----------|---------|------|
| Diet | 0.6656 | 0.4400 | ns |
| Route | 37.4846 | 0.0001 | **** |
| Time_point | 211.6458 | 0.0010 | *** |
| Diet:Route | 0.0973 | 0.7640 | ns |
| Diet:Time_point | 2.7393 | 0.1340 | ns |
| Route:Time_point | 11.3037 | 0.0080 | ** |
| Diet:Route:Time_point | 0.3065 | 0.5960 | ns |

We looked for main effects by splitting the data by each predictor separately and analyzed the respective remaining two predictor by a Robust two-way ANOVA. After adjustment of p-values for multiple tests, we observed statistically significant differences for the “Time_point” variable, when the data was split by “Route” or by “Diet”, for the well-nourished group for Route, Time_point and their interaction were

statistically meaningful, while for the malnourished state, there was no meaningful interaction between these predictors when the data was split by “Diet”, and “Route” was only meaningful at 24 h post infection, when the data was split by Time_point (Appendix table 39).

Appendix Table 39
Neutrophils: Robust two-way ANOVA

| Grouper | Predictor | value | p.value | Sig. | p.value.adj | sig. |
|-----------------------|------------------|----------|---------|------|-------------|------|
| Grouped by Route | | | | | | |
| Needle | Diet | 0.2902 | 0.603 | ns | 0.6784 | ns |
| Needle | Time_point | 68.5031 | 0.001 | *** | 0.0026 | ** |
| Needle | Diet:Time_point | 1.2155 | 0.297 | ns | 0.3819 | ns |
| SF | Diet | 0.0001 | 0.993 | ns | 0.9930 | ns |
| SF | Time_point | 159.7806 | 0.001 | *** | 0.0026 | ** |
| SF | Diet:Time_point | 3.4747 | 0.086 | + | 0.1548 | ns |
| Grouped by Diet | | | | | | |
| WN | Route | 55.4130 | 0.001 | *** | 0.0026 | ** |
| WN | Time_point | 479.7172 | 0.001 | *** | 0.0026 | ** |
| WN | Route:Time_point | 31.7623 | 0.001 | *** | 0.0026 | ** |
| MN | Route | 12.4507 | 0.003 | ** | 0.0068 | ** |
| MN | Time_point | 48.2204 | 0.001 | *** | 0.0026 | ** |
| MN | Route:Time_point | 2.8633 | 0.110 | ns | 0.1800 | ns |
| Grouped by Time_point | | | | | | |
| 24_h | Diet | 3.6650 | 0.085 | + | 0.1548 | ns |
| 24_h | Route | 57.8683 | 0.001 | *** | 0.0026 | ** |
| 24_h | Diet:Route | 0.0452 | 0.837 | ns | 0.8862 | ns |
| 72_h | Diet | 1.3260 | 0.272 | ns | 0.3766 | ns |
| 72_h | Route | 2.4858 | 0.140 | ns | 0.2100 | ns |
| 72_h | Diet:Route | 0.4236 | 0.528 | ns | 0.6336 | ns |

For the analysis of the simple simple main effect for each respective predictor, we performed Robust one-way ANOVA with individual predictors of the data split by the other two predictors. The output showed “Time_point” post infestation was statistically significant across the board, while “Route” was only statistically significant during the 24 h time point. “Diet” played not significant role as a predictor (Appendix table 40).

Appendix Table 40
Neutrophils: Robust one-way ANOVA

| Predictor_1 | Predictor_2 | Effect | test | df1 | df2 | p.value | effsize | p.value.adj | sig. |
|-----------------------|-------------|------------|----------|-----|--------|---------|---------|-------------|------|
| Predictor: Time_point | | | | | | | | | |
| Needle | WN | Time_point | 203.3793 | 1 | 5.9585 | <0.0001 | 1.1183 | <0.0001 | **** |
| Needle | MN | Time_point | 14.4271 | 1 | 7.3720 | 0.0061 | 1.0154 | 0.0122 | * |
| SF | WN | Time_point | 280.9801 | 1 | 5.5483 | <0.0001 | 1.1235 | <0.0001 | **** |
| SF | MN | Time_point | 35.7185 | 1 | 7.8502 | 0.0004 | 1.0791 | 0.0011 | ** |
| Predictor: Diet | | | | | | | | | |
| 24_h | Needle | Diet | 1.9911 | 1 | 4.5651 | 0.2226 | 0.7259 | 0.2945 | ns |
| 24_h | SF | Diet | 1.7774 | 1 | 4.5402 | 0.2454 | 0.5247 | 0.2945 | ns |
| 72_h | Needle | Diet | 0.1201 | 1 | 5.1645 | 0.7426 | 0.2162 | 0.7426 | ns |
| 72_h | SF | Diet | 1.6983 | 1 | 6.9708 | 0.2339 | 0.7251 | 0.2945 | ns |
| Predictor: Route | | | | | | | | | |
| 24_h | WN | Route | 236.8953 | 1 | 7.5189 | <0.0001 | 1.1347 | <0.0001 | **** |
| 24_h | MN | Route | 14.6124 | 1 | 7.4408 | 0.0058 | 1.0516 | 0.0122 | * |
| 72_h | WN | Route | 0.9975 | 1 | 7.0332 | 0.3510 | 0.4008 | 0.3829 | ns |
| 72_h | MN | Route | 1.5798 | 1 | 7.8076 | 0.2451 | 0.5136 | 0.2945 | ns |

For the pairwise comparison, we applied a Linear contrast expression. As all three factors were dichotomous, the pairwise comparison reflected the one-way ANOVA results (Appendix table 41).

Appendix Table 41
Neutrophils: Pairwise comparison by Linear Contrast Expression

| Predictor_1 | Predictor_2 | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------------------|-------------|--------|---------|---------|----------|----------|---------|------|
| Predictor: Time_point | | | | | | | | |
| Needle | WN | 24_h | 72_h | 2.7044 | 2.2396 | 3.1692 | <0.0001 | **** |
| Needle | MN | 24_h | 72_h | 2.0686 | 0.7938 | 3.3433 | 0.0061 | ** |
| SF | WN | 24_h | 72_h | 4.5783 | 3.8966 | 5.2600 | <0.0001 | **** |
| SF | MN | 24_h | 72_h | 3.4015 | 2.0847 | 4.7184 | 0.0004 | *** |
| Predictor: Diet | | | | | | | | |
| 24_h | Needle | WN | MN | 0.4732 | -0.4142 | 1.3606 | 0.2226 | ns |
| 24_h | SF | WN | MN | 0.5915 | -0.5846 | 1.7676 | 0.2454 | ns |
| 72_h | Needle | WN | MN | -0.1626 | -1.3570 | 1.0319 | 0.7426 | ns |
| 72_h | SF | WN | MN | -0.5853 | -1.6481 | 0.4776 | 0.2339 | ns |
| Predictor: Route | | | | | | | | |
| 24_h | WN | Needle | SF | -2.1745 | -2.5040 | -1.8451 | <0.0001 | **** |

| | | | | | | | | |
|------|----|--------|----|---------|---------|---------|--------|----|
| 24_h | MN | Needle | SF | -2.0563 | -3.3131 | -0.7994 | 0.0058 | ** |
| 72_h | WN | Needle | SF | -0.3006 | -1.0117 | 0.4104 | 0.3510 | ns |
| 72_h | MN | Needle | SF | -0.7233 | -2.0561 | 0.6095 | 0.2451 | ns |

Monocytes

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the Monocytes dataset to determine the effects of “Diet”, infection “Route” and time post infection (“Time_point”) on the Monocytes counts per pooled ear single-cell suspension (Appendix table 42). As for Myeloid_cells and Neutrophils counts, “Route” and “Time_point” were statistically significant predictors. However, no significant interaction between predictors was observed for Monocytes counts.

Appendix Table 42
Monocytes: Robust three-way ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|---------|---------|------|
| Diet | 1.1474 | 0.360 | ns |
| Route | 20.1101 | 0.006 | ** |
| Time_point | 24.8237 | 0.003 | ** |
| Diet:Route | 0.8115 | 0.428 | ns |
| Diet:Time_point | 0.0604 | 0.822 | ns |
| Route:Time_point | 4.6317 | 0.096 | + |
| Diet:Route:Time_point | 2.4210 | 0.200 | ns |

We looked for main effects by splitting the data by each predictor separately and analyzed the respective remaining two predictor by a Robust two-way ANOVA. After adjustment of p-values for multiple tests, we observed statistically significant differences for the “Time_point” variable, when the data was split by “Route” or by “Diet”; in well-nourished and malnourished groups we observed statistical significance for “Route” and “Time_point”, while no significant interactions were observed when the data was split by “Diet”; and for “Route” was meaningful at both, 24 h and 72 h post infection, when the data was split by Time_point (Appendix table 43). Overall, “Diet” played no meaningful role as a predictor.

Appendix Table 43
Monocytes: Robust two-way ANOVA

| Group | Predictor | value | p.value | Sig. | p.value.adj | sig. |
|------------------|-----------------|---------|---------|------|-------------|------|
| Grouped by Route | | | | | | |
| Needle | Diet | 0.0991 | 0.760 | ns | 0.7600 | ns |
| Needle | Time_point | 25.6851 | 0.001 | *** | 0.0045 | ** |
| Needle | Diet:Time_point | 5.7556 | 0.034 | * | 0.0680 | + |
| SF | Diet | 0.6918 | 0.437 | ns | 0.4916 | ns |

| | | | | | | |
|-----------------------|------------------|---------|-------|-----|--------|----|
| SF | Time_point | 23.4448 | 0.001 | *** | 0.0045 | ** |
| SF | Diet:Time_point | 1.2852 | 0.298 | ns | 0.3831 | ns |
| Grouped by Diet | | | | | | |
| WN | Route | 47.9950 | 0.001 | *** | 0.0045 | ** |
| WN | Time_point | 68.8826 | 0.001 | *** | 0.0045 | ** |
| WN | Route:Time_point | 1.9584 | 0.189 | ns | 0.2835 | ns |
| MN | Route | 11.5996 | 0.010 | ** | 0.0225 | * |
| MN | Time_point | 12.2280 | 0.009 | ** | 0.0225 | * |
| MN | Route:Time_point | 6.4702 | 0.038 | * | 0.0684 | + |
| Grouped by Time_point | | | | | | |
| 24_h | Diet | 0.3760 | 0.562 | ns | 0.5951 | ns |
| 24_h | Route | 20.4756 | 0.002 | ** | 0.0060 | ** |
| 24_h | Diet:Route | 1.8171 | 0.223 | ns | 0.3088 | ns |
| 72_h | Diet | 0.8951 | 0.373 | ns | 0.4476 | ns |
| 72_h | Route | 18.2554 | 0.002 | ** | 0.0060 | ** |
| 72_h | Diet:Route | 3.2974 | 0.105 | ns | 0.1718 | ns |

For the analysis of the simple simple main effect for each respective predictor, we performed Robust one-way ANOVA with individual predictors of the data split by the other two predictors. The output showed “Time_point” post infestation was statistically significant across the board with the exception for malnourished individuals inoculated by needle, while “Route” was only statistically significant during the within the well-nourished group at either time point. “Diet” was only statistically significant for the needle inoculated groups at 72 h post infection (Appendix table 44).

Appendix Table 44
Monocytes: Robust one-way ANOVA

| Predictor_1 | Predictor_2 | Effect | test | df1 | df2 | p.value | effsize | p.value.adj | sig. |
|-----------------------|-------------|------------|---------|-----|--------|---------|---------|-------------|------|
| Predictor: Time_point | | | | | | | | | |
| Needle | WN | Time_point | 24.2428 | 1 | 4.4410 | 0.0060 | 1.0016 | 0.0181 | * |
| Needle | MN | Time_point | 4.1902 | 1 | 6.5974 | 0.0823 | 0.7082 | 0.1235 | ns |
| SF | WN | Time_point | 46.2024 | 1 | 5.9611 | 0.0005 | 1.1175 | 0.0031 | ** |
| SF | MN | Time_point | 9.6448 | 1 | 4.8214 | 0.0280 | 0.9127 | 0.0528 | + |
| Predictor: Diet | | | | | | | | | |
| 24_h | Needle | Diet | 1.2696 | 1 | 7.4413 | 0.2949 | 0.4586 | 0.3538 | ns |
| 24_h | SF | Diet | 1.0760 | 1 | 4.5597 | 0.3514 | 0.4648 | 0.3834 | ns |
| 72_h | Needle | Diet | 12.7301 | 1 | 5.9683 | 0.0119 | 0.8820 | 0.0286 | * |
| 72_h | SF | Diet | 0.2225 | 1 | 5.3766 | 0.6557 | 0.3242 | 0.6557 | ns |

Predictor: Route

| | | | | | | | | | |
|------|----|-------|---------|---|--------|--------|--------|--------|----|
| 24_h | WN | Route | 19.9581 | 1 | 7.9596 | 0.0021 | 1.1520 | 0.0085 | ** |
| 24_h | MN | Route | 9.8710 | 1 | 4.3690 | 0.0308 | 0.8864 | 0.0528 | + |
| 72_h | WN | Route | 58.1556 | 1 | 5.8388 | 0.0003 | 1.1400 | 0.0031 | ** |
| 72_h | MN | Route | 1.7950 | 1 | 5.2796 | 0.2351 | 0.5765 | 0.3135 | ns |

For the pairwise comparison, we applied a Linear contrast expression. As all three factors were dichotomous, the pairwise comparison reflected the one-way ANOVA results (Appendix table 45).

Appendix Table 45
Monocytes: Pairwise comparison by Linear Contrast Expression

| Predictor_1 | Predictor_2 | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------------------|-------------|--------|---------|--------|-----------|----------|---------|------|
| Predictor: Time_point | | | | | | | | |
| Needle | WN | 24_h | 72_h | 99.6 | 45.5687 | 153.6313 | 0.0060 | ** |
| Needle | MN | 24_h | 72_h | 35.6 | -6.0383 | 77.2383 | 0.0823 | + |
| SF | WN | 24_h | 72_h | 140.0 | 89.5221 | 190.4779 | 0.0005 | *** |
| SF | MN | 24_h | 72_h | 225.6 | 36.7654 | 414.4346 | 0.0280 | * |
| Predictor: Diet | | | | | | | | |
| 24_h | Needle | WN | MN | 27.8 | -29.8466 | 85.4466 | 0.2949 | ns |
| 24_h | SF | WN | MN | -74.2 | -263.5526 | 115.1526 | 0.3514 | ns |
| 72_h | Needle | WN | MN | -36.2 | -61.0582 | -11.3418 | 0.0119 | * |
| 72_h | SF | WN | MN | 11.4 | -49.4453 | 72.2453 | 0.6557 | ns |
| Predictor: Route | | | | | | | | |
| 24_h | WN | Needle | SF | -120.2 | -182.2997 | -58.1003 | 0.0021 | ** |
| 24_h | MN | Needle | SF | -222.2 | -412.1896 | -32.2104 | 0.0308 | * |
| 72_h | WN | Needle | SF | -79.8 | -105.5774 | -54.0226 | 0.0003 | *** |
| 72_h | MN | Needle | SF | -32.2 | -93.0104 | 28.6104 | 0.2351 | ns |

Statistical power

Considering the small group $N=5$, it stood to reason that the study design was statistically underpowered by necessity to keep animal number and costs manageable. Thus, we performed a retrospective power analysis on the data by cell-type group to explore this.

Effect size estimation based on partial η^2

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 46 to 48 show the respective effect sizes. Note that upper ends of the confidence intervals

were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial eta² values from the effect size calculation were then used to the retrospective power calculations.

Appendix Table 46
Myeloid cells: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size |
|-----------------------|--------------|--------|---------|-------------|
| Diet | 0.0125 | 0.0000 | 1 | very small |
| Route | 0.6490 | 0.4742 | 1 | large |
| Time_point | 0.3162 | 0.1108 | 1 | large |
| Diet:Route | 0.0001 | 0.0000 | 1 | very small |
| Diet:Time_point | 0.0965 | 0.0000 | 1 | small |
| Route:Time_point | 0.2078 | 0.0382 | 1 | medium |
| Diet:Route:Time_point | 0.0001 | 0.0000 | 1 | very small |

Appendix Table 47
Neutrophils: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size |
|-----------------------|--------------|--------|---------|-------------|
| Diet | 0.0330 | 0.0000 | 1 | small |
| Route | 0.5814 | 0.3864 | 1 | large |
| Time_point | 0.7717 | 0.6469 | 1 | large |
| Diet:Route | 0.0117 | 0.0000 | 1 | very small |
| Diet:Time_point | 0.0758 | 0.0000 | 1 | small |
| Route:Time_point | 0.5408 | 0.3368 | 1 | large |
| Diet:Route:Time_point | 0.0223 | 0.0000 | 1 | small |

Appendix Table 48
Monocytes: Effect size estimation

| Parameter | Eta2_partial | CI_low | CI_high | Effect_size |
|-----------------------|--------------|--------|---------|-------------|
| Diet | 0.0241 | 0.0000 | 1 | small |
| Route | 0.5015 | 0.2911 | 1 | large |
| Time_point | 0.5500 | 0.3478 | 1 | large |
| Diet:Route | 0.0142 | 0.0000 | 1 | very small |
| Diet:Time_point | 0.0023 | 0.0000 | 1 | very small |
| Route:Time_point | 0.2055 | 0.0370 | 1 | medium |
| Diet:Route:Time_point | 0.0983 | 0.0000 | 1 | small |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power in once data. For small mean differences within data with a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. For Myeloid_cells, the predictors “Route” and “Time_point” as much as the interaction “Diet:Time_point” resulted in optimal sample sizes that were within the total sample size of $N=40, 40, 40$ (Appendix table 49). The large proposed sample sizes for “Diet” on its own and within most of its interactions suggested little statistically significant difference between groups with respect to “Diet”.

Appendix Table 49
Myeloid Cells: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|----------|
| Diet | | | | | |
| Diet | 0.8 | 620 | 7.873 | 1 | 617.5 |
| Route | | | | | |
| Route | 0.8 | 7 | 12.601 | 1 | 4.815 |
| Time_point | | | | | |
| Time_point | 0.8 | 20 | 8.832 | 1 | 17.095 |
| Diet:Route | | | | | |
| Diet | 0.8 | 64408 | 7.849 | 1 | 64403.09 |
| Route | 0.8 | 64408 | 7.849 | 1 | 64403.09 |
| Diet:Route | 0.8 | 64408 | 7.849 | 1 | 64403.09 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 76 | 8.065 | 1 | 71.503 |
| Time_point | 0.8 | 76 | 8.065 | 1 | 71.503 |
| Diet:Time_point | 0.8 | 76 | 8.065 | 1 | 71.503 |
| Route:Time_point | | | | | |
| Route | 0.8 | 33 | 8.422 | 1 | 28.106 |
| Time_point | 0.8 | 33 | 8.422 | 1 | 28.106 |
| Route:Time_point | 0.8 | 33 | 8.422 | 1 | 28.106 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 83400 | 7.849 | 1 | 83391.34 |
| Route | 0.8 | 83400 | 7.849 | 1 | 83391.34 |
| Time_point | 0.8 | 83400 | 7.849 | 1 | 83391.34 |
| Diet:Route | 0.8 | 83400 | 7.849 | 1 | 83391.34 |

| | | | | | |
|-----------------------|-----|-------|-------|---|----------|
| Diet:Time_point | 0.8 | 83400 | 7.849 | 1 | 83391.34 |
| Route:Time_point | 0.8 | 83400 | 7.849 | 1 | 83391.34 |
| Diet:Route:Time_point | 0.8 | 83400 | 7.849 | 1 | 83391.34 |

Similar observation were made for Neutrophils counts, but here the interaction “Route:Time_point” was within the total sample size of $N=40, 40, 40$ (Appendix Table 50). The involvement of “Diet” produced large minimum sample sizes again, suggesting that no true difference may be observed here with respect to “Diet”.

Appendix Table 50
Neutrophils: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|---------|
| Diet | | | | | |
| Diet | 0.8 | 232 | 7.915 | 1 | 229.662 |
| Route | | | | | |
| Route | 0.8 | 9 | 11.256 | 1 | 6.104 |
| Time_point | | | | | |
| Time_point | 0.8 | 6 | 17.344 | 1 | 3.132 |
| Diet:Route | | | | | |
| Diet | 0.8 | 665 | 7.872 | 1 | 660.599 |
| Route | 0.8 | 665 | 7.872 | 1 | 660.599 |
| Diet:Route | 0.8 | 665 | 7.872 | 1 | 660.599 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 98 | 8.013 | 1 | 93.677 |
| Time_point | 0.8 | 98 | 8.013 | 1 | 93.677 |
| Diet:Time_point | 0.8 | 98 | 8.013 | 1 | 93.677 |
| Route:Time_point | | | | | |
| Route | 0.8 | 10 | 11.518 | 1 | 5.78 |
| Time_point | 0.8 | 10 | 11.518 | 1 | 5.78 |
| Route:Time_point | 0.8 | 10 | 11.518 | 1 | 5.78 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 347 | 7.894 | 1 | 338.278 |
| Route | 0.8 | 347 | 7.894 | 1 | 338.278 |
| Time_point | 0.8 | 347 | 7.894 | 1 | 338.278 |
| Diet:Route | 0.8 | 347 | 7.894 | 1 | 338.278 |

| | | | | | |
|-----------------------|-----|-----|-------|---|---------|
| Diet:Time_point | 0.8 | 347 | 7.894 | 1 | 338.278 |
| Route:Time_point | 0.8 | 347 | 7.894 | 1 | 338.278 |
| Diet:Route:Time_point | 0.8 | 347 | 7.894 | 1 | 338.278 |

For Monocytes counts, the same observations were true as for Myeloid_cells counts with the exception that “Diet” did not have a major detrimental effect on predicted minimum sample size for the three-way interaction (“Diet:Route:Time_point”; Appendix Table 51).

Appendix Table 51
Monocytes: Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|----------|
| Diet | | | | | |
| Diet | 0.8 | 320 | 7.897 | 1 | 317.655 |
| Route | | | | | |
| Route | 0.8 | 11 | 10.197 | 1 | 8.134 |
| Time_point | | | | | |
| Time_point | 0.8 | 9 | 10.786 | 1 | 6.826 |
| Diet:Route | | | | | |
| Diet | 0.8 | 547 | 7.877 | 1 | 542.2 |
| Route | 0.8 | 547 | 7.877 | 1 | 542.2 |
| Diet:Route | 0.8 | 547 | 7.877 | 1 | 542.2 |
| Diet:Time_point | | | | | |
| Diet | 0.8 | 3455 | 7.853 | 1 | 3450.162 |
| Time_point | 0.8 | 3455 | 7.853 | 1 | 3450.162 |
| Diet:Time_point | 0.8 | 3455 | 7.853 | 1 | 3450.162 |
| Route:Time_point | | | | | |
| Route | 0.8 | 33 | 8.413 | 1 | 28.521 |
| Time_point | 0.8 | 33 | 8.413 | 1 | 28.521 |
| Route:Time_point | 0.8 | 33 | 8.413 | 1 | 28.521 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.8 | 75 | 8.083 | 1 | 66.118 |
| Route | 0.8 | 75 | 8.083 | 1 | 66.118 |
| Time_point | 0.8 | 75 | 8.083 | 1 | 66.118 |
| Diet:Route | 0.8 | 75 | 8.083 | 1 | 66.118 |
| Diet:Time_point | 0.8 | 75 | 8.083 | 1 | 66.118 |

| | | | | | |
|-----------------------|-----|----|-------|---|--------|
| Route:Time_point | 0.8 | 75 | 8.083 | 1 | 66.118 |
| Diet:Route:Time_point | 0.8 | 75 | 8.083 | 1 | 66.118 |

Retrospective calculation of statistical power in our data analysis

The observations from the retrospective minimum sample size calculation was reflected in the power calculation for our data. For Myeloid_cells counts, only “Route” and “Time_point” and the interaction of “Diet:Time_point” had sufficient statistical power to have a change to find statistically meaning differences in the data (Appendix Table 52).

Appendix Table 52
Myeloid Cells: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|-----|
| Diet | | | | | |
| Diet | 0.107 | 40 | 0.508 | 1 | 38 |
| Route | | | | | |
| Route | 1 | 40 | 73.967 | 1 | 38 |
| Time_point | | | | | |
| Time_point | 0.987 | 40 | 18.501 | 1 | 38 |
| Diet:Route | | | | | |
| Diet | 0.051 | 40 | 0.005 | 1 | 36 |
| Route | 0.051 | 40 | 0.005 | 1 | 36 |
| Diet:Route | 0.051 | 40 | 0.005 | 1 | 36 |
| Diet:Time_point | | | | | |
| Diet | 0.521 | 40 | 4.273 | 1 | 36 |
| Time_point | 0.521 | 40 | 4.273 | 1 | 36 |
| Diet:Time_point | 0.521 | 40 | 4.273 | 1 | 36 |
| Route:Time_point | | | | | |
| Route | 0.883 | 40 | 10.492 | 1 | 36 |
| Time_point | 0.883 | 40 | 10.492 | 1 | 36 |
| Route:Time_point | 0.883 | 40 | 10.492 | 1 | 36 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.05 | 40 | 0.004 | 1 | 32 |
| Route | 0.05 | 40 | 0.004 | 1 | 32 |
| Time_point | 0.05 | 40 | 0.004 | 1 | 32 |

| | | | | | |
|-----------------------|------|----|-------|---|----|
| Diet:Route | 0.05 | 40 | 0.004 | 1 | 32 |
| Diet:Time_point | 0.05 | 40 | 0.004 | 1 | 32 |
| Route:Time_point | 0.05 | 40 | 0.004 | 1 | 32 |
| Diet:Route:Time_point | 0.05 | 40 | 0.004 | 1 | 32 |

For Neutrophils counts, “Route” and “Time_points as much as their interaction had >80% statistical power, while the interaction”Diet:Time_point” was close to 80% statistical power (Appendix Table 53).

Appendix Table 53
Neutrophils: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|---------|-----|-----|
| Diet | | | | | |
| Diet | 0.207 | 40 | 1.367 | 1 | 38 |
| Route | | | | | |
| Route | 1 | 40 | 55.554 | 1 | 38 |
| Time_point | | | | | |
| Time_point | 1 | 40 | 135.188 | 1 | 38 |
| Diet:Route | | | | | |
| Diet | 0.103 | 40 | 0.474 | 1 | 36 |
| Route | 0.103 | 40 | 0.474 | 1 | 36 |
| Diet:Route | 0.103 | 40 | 0.474 | 1 | 36 |
| Diet:Time_point | | | | | |
| Diet | 0.422 | 40 | 3.281 | 1 | 36 |
| Time_point | 0.422 | 40 | 3.281 | 1 | 36 |
| Diet:Time_point | 0.422 | 40 | 3.281 | 1 | 36 |
| Route:Time_point | | | | | |
| Route | 1 | 40 | 47.107 | 1 | 36 |
| Time_point | 1 | 40 | 47.107 | 1 | 36 |
| Route:Time_point | 1 | 40 | 47.107 | 1 | 36 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.153 | 40 | 0.912 | 1 | 32 |
| Route | 0.153 | 40 | 0.912 | 1 | 32 |
| Time_point | 0.153 | 40 | 0.912 | 1 | 32 |
| Diet:Route | 0.153 | 40 | 0.912 | 1 | 32 |
| Diet:Time_point | 0.153 | 40 | 0.912 | 1 | 32 |

| | | | | | |
|-----------------------|-------|----|-------|---|----|
| Route:Time_point | 0.153 | 40 | 0.912 | 1 | 32 |
| Diet:Route:Time_point | 0.153 | 40 | 0.912 | 1 | 32 |

Similar to Neutrophils counts, for Monocytes counts, “Route” and “Time_points as much as their interaction had >80% statistical power, while the interaction”Diet:Time_point” was close to 80% statistical power (Appendix Table 54).

Appendix Table 54
Monocytes: Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|-----------------------|-------|---------|--------|-----|-----|
| Diet | | | | | |
| Diet | 0.163 | 40 | 0.988 | 1 | 38 |
| Route | | | | | |
| Route | 1 | 40 | 40.247 | 1 | 38 |
| Time_point | | | | | |
| Time_point | 1 | 40 | 48.886 | 1 | 38 |
| Diet:Route | | | | | |
| Diet | 0.115 | 40 | 0.577 | 1 | 36 |
| Route | 0.115 | 40 | 0.577 | 1 | 36 |
| Diet:Route | 0.115 | 40 | 0.577 | 1 | 36 |
| Diet:Time_point | | | | | |
| Diet | 0.06 | 40 | 0.091 | 1 | 36 |
| Time_point | 0.06 | 40 | 0.091 | 1 | 36 |
| Diet:Time_point | 0.06 | 40 | 0.091 | 1 | 36 |
| Route:Time_point | | | | | |
| Route | 0.879 | 40 | 10.347 | 1 | 36 |
| Time_point | 0.879 | 40 | 10.347 | 1 | 36 |
| Route:Time_point | 0.879 | 40 | 10.347 | 1 | 36 |
| Diet:Route:Time_point | | | | | |
| Diet | 0.526 | 40 | 4.362 | 1 | 32 |
| Route | 0.526 | 40 | 4.362 | 1 | 32 |
| Time_point | 0.526 | 40 | 4.362 | 1 | 32 |
| Diet:Route | 0.526 | 40 | 4.362 | 1 | 32 |
| Diet:Time_point | 0.526 | 40 | 4.362 | 1 | 32 |
| Route:Time_point | 0.526 | 40 | 4.362 | 1 | 32 |

Conclusion

In conclusion, it can be said that the infection route (needle, sand fly, or uninfected) as much as the time point of data collection (24 h vs. 72 h) were the primary difference makers with respect to observed IL-1 β ⁺ Myeloid_cells, Neutrophils and Monocytes counts in pooled mouse ear single-cell suspensions. It is of note that the nutritional status of the individuals did not have a detectable impact on the observed IL-1 β ⁺ cell counts in the site of infection.

Panel h

Here, we are presenting the relative fold difference in the heme oxygenase-1 (HO-1) protein levels in well-nourished (WN) and malnourished (MN) BALB/s mice infected with *Leishmania donovani* parasites via the sand fly route or uninfected. Heat-shock protein 90 (Hsp90) was used as a house-keeping gene control for the Western blot loading control and well-nourished control (WN Ctrl) mice served as a HO-1 concentration reference. Three Western blots were produced with different pooled samples as biological replicas. To normalize the band intensity readings, we first calculated the normalization factor by dividing the Hsp90 value from the WN Ctrl sample of one blot with the Hsp90 values of all other line on all blots. Then we multiplied the HO-1 readings by the normalized Hsp90 readings for each sample lane, respectively. Fold change differences were then calculated by dividing all normalized HO-1 readings with the normalized WN Ctrl HO-1 reading for each Western blot, respectively. This resulted in all WN Ctrl HO-1 readings to be set to 1. As this eliminated any data variance in WN Ctrl group, it was treated as the baseline reference and thus, was disconsidered from the statistical analysis.

The remaining three groups, well-nourished sand fly infected (WN SF), malnourished control (MN Ctrl) and malnourished sand fly infected (MN SF) were analyzed by the Kruskal-Wallis test, were analyzed by Kruskal-Wallis test followed post hoc by the Dunn's test for pairwise comparison. The output showed a p-value of 0.148, which was not statistically significant. The pairwise comparison by Dunn's test confirmed that no statistically significant differences were observed between WN SF, MN Ctrl and MN SF.

Appendix Table 55
Dunn's test

| group1 | group2 | n1 | n2 | statistic | p | p.adj | p.adj.signif |
|---------|--------|----|----|-----------|--------|--------|--------------|
| MN_CTRL | MN_SF | 3 | 3 | 1.9379 | 0.0526 | 0.1579 | ns |
| MN_CTRL | WN_SF | 3 | 3 | 0.7454 | 0.4561 | 0.4661 | ns |
| MN_SF | WN_SF | 3 | 3 | -1.1926 | 0.2330 | 0.4661 | ns |

It is of not that the median fold difference of the MN Ctrl group was 2.766 higher than that of the WN Ctrl group, showing that more HO-1 was present in malnourished mice prior to infection, but that did not seem

to have a profound impact on the median HO-1 fold differences compared to the WN Ctrl reference post infection by sand fly for malnourished compared to well-nourished mice (WN SF: 6.379, MN SF: 8.088).

Figure 2

Panel a

Data analysis

We analysed the frequency of *Leishmania donovani* dissemination to the draining lymph node in a total of $N=32$ well-nourished (WN) and malnourished (MN) BALB/c mice infected intradermally either by “needle” injection or sand fly bite (SF) (N : MN_Needle=8, MN_SF=8, WN_Needle=8, WN_SF=8) by contingency table analysis and logistic regression.

Contingency table

Due to the small sample sizes, there were several expected counts <5 , why we opted for the Fisher’s Exact test, which had the added benefit of exact p-value calculation. The analysis rendered a p-value of 0.444, suggesting no statistically significant difference between groups. This was confirmed by the pairwise Fisher’s Exact test corrected by the Benjamin-Hochberg method (Appendix table 56).

Appendix Table 56
Pairwise Fisher’s Exact test

| group1 | group2 | n | estimate | p | conf.low | conf.high | alternative | p.adj | p.adj.signif |
|-----------|-----------|----|----------|-------|----------|-----------|-------------|-------|--------------|
| MN_Needle | MN_SF | 16 | Inf | 1.000 | 0.0256 | Inf | two.sided | 1 | ns |
| MN_Needle | WN_Needle | 16 | 0.2605 | 0.569 | 0.0040 | 4.4010 | two.sided | 1 | ns |
| MN_Needle | WN_SF | 16 | 0.4516 | 1.000 | 0.0064 | 10.7913 | two.sided | 1 | ns |
| MN_SF | WN_Needle | 16 | 0.0000 | 0.200 | 0.0000 | 2.2053 | two.sided | 1 | ns |
| MN_SF | WN_SF | 16 | 0.0000 | 0.467 | 0.0000 | 5.2059 | two.sided | 1 | ns |
| WN_Needle | WN_SF | 16 | 1.7346 | 1.000 | 0.1359 | 28.9942 | two.sided | 1 | ns |

We observed a 3.84-fold and a 2.21-fold reduction in parasite dissemination events in well-nourished animals infected by needle and sand fly, respectively, compared to malnourished, needle inoculated ones, but the 95% confidence intervals were so large that 1 was included, suggesting that this decreased occurrence in dissemination was not statistically significant (Appendix table 57). However, applying a retrospective statistical power calculation showed that the sample size was too small to detect a meaningful difference here and thus, our statistical power was well below the standard 80% (Appendix table 58), but larger sample sizes were prohibitive due to cost and loss of life.

Appendix Table 57
Odds Ratios

| Groups | estimate | lower | upper | p.value |
|-----------|----------|--------|---------|---------|
| MN_Needle | 1.0000 | NA | NA | NA |
| MN_SF | Inf | 0.0256 | Inf | 1.0000 |
| WN_Needle | 0.2605 | 0.0040 | 4.4010 | 0.5692 |
| WN_SF | 0.4516 | 0.0064 | 10.7913 | 1.0000 |

Appendix Table 58
Retrospective Power Calculation

| Parameters | Calculation for | |
|--------------------------|-----------------|-------------------|
| | Sample size | Statistical power |
| Statistical power | 0.8 | 0.367 |
| Total n | 86 | 32 |
| Degrees of freedom | 3 | 3 |
| Non-centrality parameter | 10.903 | 4.103 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.633 |

Logistic regression

We applied a logistic regression model to the same data and assessed the two predictor variables “Diet” and “Route” without an interaction term to assess individual predictor contribution to the outcome. The data output showed that infection route did not have much impact on whether parasites made it to the draining lymph nodes or not ($p=0.3472$), suggesting a mere 2.57-fold increase in probability of parasite dissemination when sand flies were used (Appendix table 59), which was equivalent to a small effect size (Appendix table 60). Conversely, although not reaching statistical significance either, there was an indication in the data, that “Diet” affects parasites capacity to disseminate to the draining lymph nodes as the p-value approached statistical significance ($p=0.0949$), indicating a 7.19-fold increase in the probability of parasite dissemination (Appendix table 59), which was equivalent to a large effect size (Appendix table 60). Even so, neither predictor achieved statistical significance according to Wald test (Appendix table 61).

Appendix Table 59
Logistic regression output

| Groups | Estimate | lower CI | upper CI | Std. Error | partial.R2 | z value | Pr(> z) | sig. |
|-------------|----------|----------|----------|------------|------------|---------|----------|------|
| (Intercept) | 0.3582 | -0.9967 | 1.7994 | 0.6888 | 0.0000 | 0.5200 | 0.6030 | ns |

| | | | | | | | | |
|---------|--------|---------|--------|--------|--------|--------|--------|----|
| DietMN | 1.9727 | -0.0533 | 5.0210 | 1.1813 | 0.1206 | 1.6699 | 0.0949 | + |
| RouteSF | 0.9452 | -0.9604 | 3.1423 | 1.0055 | 0.0340 | 0.9400 | 0.3472 | ns |

Appendix Table 60
Odds Ratios

| Predictor | OR | 2.5 % | 97.5 % | Effect_size |
|-------------|--------|--------|----------|-------------|
| (Intercept) | 1.4307 | 0.3691 | 6.0459 | very small |
| DietMN | 7.1900 | 0.9481 | 151.5553 | large |
| RouteSF | 2.5733 | 0.3827 | 23.1574 | small |

Appendix Table 61
Wald test

| Predictor | chi2 | df | P |
|-----------|------|----|--------|
| Diet | 2.79 | 1 | 0.0949 |
| Route | 0.88 | 1 | 0.3472 |

However, although none of the predictors was statistically significant in the logistic regression model, thus did not mean that they had no meaningful biological effect. A retrospective sample size and power calculation with the study data showed that the study was well underpowered for the logistic regression, as it was already for the contingency table analysis (Appendix table 62). The proposed minimum total sample size that would have given both predictor a chance to identify a meaningful statistical different by this calculation was 382, which was ~12-times of the study's sample size, which was prohibitive due to cost and excessive loss of life. Even so, there was a good indication of potential biological significance with respect to nutritional status. Considering the predicted probability of parasite dissemination, it can be seen that being malnourished increased the probability of parasite dissemination (Appendix table 63). The large confidence intervals, however, did not render statistical significance, which does not exclude biological significance. Even the route of infection showed at least in the well-nourished model a considerable increase in predicted probability of parasite dissemination from needle to sand fly inoculation. Thus, the lack of statistical power and high data variance prevented the obtainment of statistical significance.

Appendix Table 62
Retrospective power analyses

| Calculation | Predictor | Beta0 | Beta1 | R-square | alpha | Power | TotalN | NCP | Alternative |
|-------------|-----------|-------|-------|----------|-------|-------------|------------|-------|-------------|
| Sample_size | Diet | 1.466 | 1.973 | 0.034 | 0.05 | 0.80 | 144 | 2.606 | not equal |
| Sample_size | Route | 1.466 | 0.945 | 0.121 | 0.05 | 0.80 | 382 | 2.752 | not equal |
| Power | Diet | 0.358 | 1.973 | 0.034 | 0.05 | 0.48 | 32 | 1.908 | not equal |

| | | | | | | | | | |
|-------|-------|-------|-------|-------|------|-------------|----|-------|-----------|
| Power | Route | 0.358 | 0.945 | 0.121 | 0.05 | 0.19 | 32 | 1.117 | not equal |
|-------|-------|-------|-------|-------|------|-------------|----|-------|-----------|

Appendix Table 63
Predicted probability of parasite dissemination

| Diet | Route | fit | se.fit | Predicted_Probability | lower_CI | upper_CI |
|------|--------|--------|--------|-----------------------|----------|----------|
| WN | Needle | 0.3582 | 0.6888 | 0.5886 | 0.2706 | 0.8466 |
| WN | SF | 1.3034 | 0.8107 | 0.7864 | 0.4291 | 0.9475 |
| MN | Needle | 2.3309 | 1.0825 | 0.9114 | 0.5521 | 0.9885 |
| MN | SF | 3.2761 | 1.2532 | 0.9636 | 0.6942 | 0.9968 |

Panel b

Data analysis

We analyzed the frequency of *Leishmania donovani* dissemination to the spleen in a total of $N=92$ well-nourished (WN) and malnourished (MN) BALB/c mice infected intradermally either by “needle” injection or sand fly bite (SF) (N : MN_Needle=23, MN_SF=23, WN_Needle=23, WN_SF=23) by contingency table analysis and logistic regression.

Contingency table

Here, we opted for the Chi-square test as assumptions held. The analysis rendered a p-value of 0.0742, suggesting no statistically significant difference between groups. This was confirmed by the pairwise Chi-square test corrected by the Benjamin-Hochberg method (Appendix table 64).

Appendix Table 64
Pairwise Chi-square test

| n | group1 | group2 | statistic | p | df | p.adj | p.adj.signif |
|----|-----------|-----------|-----------|--------|----|-------|--------------|
| 46 | MN_Needle | MN_SF | 3.7829 | 0.0518 | 1 | 0.155 | ns |
| 46 | MN_Needle | WN_Needle | 0.0000 | 1.0000 | 1 | 1.000 | ns |
| 46 | MN_Needle | WN_SF | 0.0000 | 1.0000 | 1 | 1.000 | ns |
| 46 | MN_SF | WN_Needle | 4.9730 | 0.0257 | 1 | 0.154 | ns |
| 46 | MN_SF | WN_SF | 2.6960 | 0.1010 | 1 | 0.202 | ns |
| 46 | WN_Needle | WN_SF | 0.1027 | 0.7490 | 1 | 1.000 | ns |

However, while we observed a 1.21-fold reduction in parasite dissemination events in well-nourished animals infected by needle, compared to malnourished, needle inoculated ones, we also observed an increase in parasite dissemination in both, malnourished and well-nourished mice, infected by sand fly bite, but only the malnourished group had significantly higher odds of dissemination (Appendix table 65).

Applying a retrospective statistical power calculation showed that the sample size was too small to detect a meaningful statistical difference here and thus, our statistical power was somewhat below the standard 80% (Appendix table 66), but larger sample sizes were prohibitive due to cost and loss of life.

Appendix Table 65
Odds Ratios

| Groups | estimate | lower | upper | p.value |
|-----------|----------|--------|----------|---------|
| MN_Needle | 1.0000 | NA | NA | NA |
| MN_SF | 8.3203 | 1.2566 | 227.4435 | 0.047 |
| WN_Needle | 0.8255 | 0.2294 | 2.9144 | 1.000 |
| WN_SF | 1.2305 | 0.3296 | 4.7180 | 1.000 |

Appendix Table 66
Retrospective Power Calculation

| Parameters | Calculation for | |
|--------------------------|-----------------|-------------------|
| | Sample size | Statistical power |
| Statistical power | 0.8 | 0.585 |
| Total n | 145 | 92 |
| Degrees of freedom | 3 | 3 |
| Non-centrality parameter | 10.903 | 6.93 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.415 |

Logistic regression

We applied a logistic regression model to the same data and assessed the two predictor variables “Diet” and “Route” without an interaction term to assess individual predictor contribution to the outcome. The data output showed that infection route had much more impact on whether parasites made it to the spleen or not ($p=0.0524$) than to the draining lymph nodes (Fig.2a). However, we observed a mere 2.76-fold increase in probability of parasite dissemination when sand flies were used for infection (Appendix table 67), which was equivalent to a small effect size (Appendix table 68). There was also little indication in the data, that “Diet” on its own affected parasite capacity to disseminate to the spleen, indicating a mere 2.15-fold increase in the probability of parasite dissemination (Appendix table 67), which was equivalent to a small effect size (Appendix table 68). Thus, neither predictor on its own achieved statistical significance according to Wald test (Appendix table 69). However, re-running the logistic regression model with an interaction term showed that the interaction between “Diet” and “Route” had much more potency than either predictor on its own, already hinted at by the odds ratios from the chi-square analysis, even though, the interaction term did not achieve statistical significance (Appendix table 70).

Appendix Table 67**Logistic regression output**

| Groups | Estimate | lower CI | upper CI | Std. Error | partial.R2 | z value | Pr(> z) | sig. |
|-------------|----------|----------|----------|------------|------------|---------|----------|------|
| (Intercept) | 0.3695 | -0.3890 | 1.1548 | 0.3897 | 0.0000 | 0.9482 | 0.3430 | ns |
| DietMN | 0.7640 | -0.2277 | 1.8143 | 0.5156 | 0.0233 | 1.4817 | 0.1384 | ns |
| RouteSF | 1.0166 | 0.0186 | 2.0970 | 0.5241 | 0.0403 | 1.9396 | 0.0524 | + |

Appendix Table 68**Odds Ratios**

| Predictor | OR | 2.5 % | 97.5 % | Effect_size |
|-------------|--------|--------|--------|-------------|
| (Intercept) | 1.4470 | 0.6778 | 3.1733 | very small |
| DietMN | 2.1470 | 0.7964 | 6.1365 | small |
| RouteSF | 2.7638 | 1.0188 | 8.1414 | small |

Appendix Table 69**Wald test**

| Predictor | chi2 | df | P |
|-----------|------|----|--------|
| Diet | 2.20 | 1 | 0.1384 |
| Route | 3.76 | 1 | 0.0524 |

Appendix Table 70**Logistic regression with interaction output**

| Groups | Estimate | Std. Error | z value | Pr(> z) |
|----------------|----------|------------|---------|----------|
| (Intercept) | 0.6286 | 0.4378 | 1.4358 | 0.1510 |
| DietMN | 0.1981 | 0.6301 | 0.3143 | 0.7533 |
| RouteSF | 0.4128 | 0.6459 | 0.6392 | 0.5227 |
| DietMN:RouteSF | 1.8515 | 1.2914 | 1.4338 | 0.1516 |

A retrospective sample size and power calculation with the study data showed that the study was well underpowered for the logistic regression, as it was already for the contingency table analysis (Appendix table 71). The proposed minimum total sample size that permit both predictor a chance to identify a meaningful statistical different by this calculation was 397, which was ~4-times of the study's sample size, which was prohibitive due to cost and excessive loss of life. Even so, there was a good indication of potential biological significance with respect to interaction between "Diet" and "Route" of infection. Considering the predicted probability of parasite dissemination, it can be seen that parasite transmission

by sand fly bite increased the predicted probability of parasite dissemination from needle inoculation for either nutritional status, respectively, which significant for well-nourished mice as can be seen from the confidence intervals (Appendix table 72). “Diet” on its own had a bigger impact on parasite dissemination for needle inoculation. Thus, there are good indications here that parasite transmission by sand fly had a meaningful biological effect on the probability of parasite dissemination to the spleen, too, which was aided by nutritional status more so for the needle inoculation than for the sand fly transmission.

Appendix Table 71
Retrospective power analyses

| Calculation | Predictor | Beta0 | Beta1 | R-square | alpha | Power | TotalN | NCP | Alternative |
|-------------|-----------|-------|-------|----------|-------|-------------|------------|-------|-------------|
| Sample_size | Diet | 1.157 | 0.764 | 0.040 | 0.05 | 0.80 | 397 | 2.773 | not equal |
| Sample_size | Route | 1.157 | 1.017 | 0.023 | 0.05 | 0.80 | 246 | 2.751 | not equal |
| Power | Diet | 0.369 | 0.764 | 0.040 | 0.05 | 0.37 | 92 | 1.641 | not equal |
| Power | Route | 0.369 | 1.017 | 0.023 | 0.05 | 0.56 | 92 | 2.114 | not equal |

Appendix Table 72
Predicted probability of parasite dissemination

| Diet | Route | fit | se.fit | Predicted_Probability | lower_CI | upper_CI |
|------|--------|--------|--------|-----------------------|----------|----------|
| WN | Needle | 0.3695 | 0.3897 | 0.5913 | 0.4027 | 0.7564 |
| WN | SF | 1.3861 | 0.4557 | 0.8000 | 0.6208 | 0.9071 |
| MN | Needle | 1.1335 | 0.4333 | 0.7565 | 0.5706 | 0.8790 |
| MN | SF | 2.1502 | 0.5268 | 0.8957 | 0.7535 | 0.9602 |

Panel c

Here, we present the parasite counts per isolated draining lymph node according to qPCR as a measure of parasite dissemination to the organ. To analyze this data, we had to re-scale it, due to the occurrence of frequent zero-values in instances of no detection, by dividing all value by the smallest non-zero value in the dataset. This resulted in a approximate Poisson / negative binomial distribution, which allowed the convenient analysis of the re-scaled and rounded counts by the appropriate models for these distributions.

We analyzed a total of $N=32$ BALB/c mice (WN_Needle=8, WN_SF=8, MN_Needle=8, MN_SF=8). These were the same mice as analyzed in figure 2a for parasite dissemination events. Here, we quantified parasite burden per isolated draining lymph node. For the data analysis we tested several Poisson and negative binomial-type regression models. Based on the Akaike information criterion (AIC) we selected a standard negative_binomial regression model for the data analysis post data re-scaling. The model fitted the data well producing no statistically significant departure from 1 for its dispersion ratio (dispersion_ratio: 0.9701, p_value: 0.632) and showing a reasonable pseudo- R^2 (Nagelkerke (Cragg and Uhler): 0.412452). The model output showed that both, “Diet” and “Route” were statistically significant predictors, but there was no statistically significant interaction between these two predictors (Appendix table 73).

Appendix Table 73
Negative binomial regression model output

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|----------------|----------|------------|---------|----------|------|
| (Intercept) | 2.7568 | 0.5315 | 5.1868 | <0.0001 | **** |
| DietWN | -1.7918 | 0.7776 | -2.3041 | 0.0212 | * |
| RouteSF | 2.0715 | 0.7470 | 2.7729 | 0.0056 | ** |
| DietWN:RouteSF | 0.5977 | 1.0762 | 0.5554 | 0.5786 | ns |

The pairwise comparison based on the estimated marginal means showed that well-nourished needle inoculated BALB/c mice had statistically significantly less parasites in the draining lymph nodes than all other groups (Appendix table 74) and clearly clustered on its own (Appendix table 75). On the other hand, malnourished needle inoculated and well-nourished sand fly transmitted infection were comparable in their degree of parasite dissemination. Also, malnourished and well-nourished sand fly transmitted infection clustered together. Together, this data suggested that sand fly transmitted infections had a statistically significantly higher degree of parasite dissemination for each nutritional state, while malnourishment also exacerbated parasite dissemination. Ultimately, the effects of both variables seemed additive, but the regression model did not support this hypothesis, suggesting that either effect acted independently of one another.

Appendix Table 74
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------------|----------|--------|-----|---------|---------|------|
| MN Needle - WN Needle | 1.7918 | 0.7776 | Inf | 2.3041 | 0.0318 | * |
| MN Needle - MN SF | -2.0715 | 0.7470 | Inf | -2.7729 | 0.0111 | * |
| WN Needle - WN SF | -2.6692 | 0.7746 | Inf | -3.4458 | 0.0017 | ** |
| MN SF - WN SF | 1.1940 | 0.7439 | Inf | 1.6050 | 0.1302 | ns |

Appendix Table 75
Pairwise comparison letter code

| Diet | Route | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|------|--------|--------|--------|-----|------------|------------|--------|
| WN | Needle | 0.9651 | 0.5676 | Inf | -0.4527 | 2.3828 | a |
| MN | Needle | 2.7568 | 0.5315 | Inf | 1.4293 | 4.0844 | b |
| WN | SF | 3.6343 | 0.5271 | Inf | 2.3177 | 4.9509 | bc |
| MN | SF | 4.8283 | 0.5249 | Inf | 3.5171 | 6.1395 | c |

Panel d

Here, we present the parasite counts per isolated spleen according to qPCR as a measure of parasite dissemination to the organ. To analyze this data, we had to re-scale it, due to the occurrence of frequent zero-values in instances of no detection, by dividing all value by the smallest non-zero value in the dataset. This resulted in a approximate Poisson / negative binomial distribution, which allowed the convenient analysis of the re-scaled and rounded counts by the appropriate models for these distributions.

We analyzed a total of $N=92$ BALB/c mice (WN_Needle=23, WN_SF=23, MN_Needle=23, MN_SF=23). These were the same mice as analyzed in figure 2b for parasite dissemination events. Here, we quantified parasite burden per isolated spleen. For the data analysis, we tested several Poisson and negative binomial-type regression models. Based on the Akaike information criterion (AIC), we selected a standard negative_binomial regression model for the data analysis. The model fit of the data was moderate, producing no statistically significant departure from 1 for its dispersion ratio (dispersion_ratio: 1.3039, p_value: 0.392), but producing only a pseudo- R^2 of 0.178589 (Nagelkerke (Cragg and Uhler)). The model output showed that “Diet” was the only statistically significant predictors, with “Route” and the interaction term producing no statistically significant result (Appendix table 76).

Appendix Table 76
Negative binomial regression model output

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|----------------|----------|------------|---------|----------|------|
| (Intercept) | 2.9095 | 0.3223 | 9.0285 | <0.0001 | **** |
| DietWN | -0.9391 | 0.4598 | -2.0424 | 0.0411 | * |
| RouteSF | 0.3943 | 0.4549 | 0.8669 | 0.3860 | ns |
| DietWN:RouteSF | -1.0005 | 0.6535 | -1.5310 | 0.1258 | ns |

The pairwise comparison based on the estimated marginal means showed that different nutritional statuses generally produced statistical significance, with the exception of malnourished and well-nourished needle inoculation, which was close to the significance threshold, though (Appendix table 77). In particular, well-nourished sand fly transmitted infection clustered away from malnourished mice, regardless of infection route (Appendix table 78). Conversely, parasite dissemination in sand fly transmitted infections in malnourished BALB/c mice were clearly more efficient than in a well-nourished setting. Contrary to the draining lymph nodes, parasite dissemination to the spleen was clearly determined more by the animals nutritional state, whether than infection route.

Appendix Table 77
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------------|----------|--------|-----|---------|---------|------|
| MN Needle - WN Needle | 0.9391 | 0.4598 | Inf | 2.0424 | 0.0617 | + |
| MN Needle - MN SF | -0.3943 | 0.4549 | Inf | -0.8669 | 0.3860 | ns |

| | | | | | | |
|-------------------|--------|--------|-----|--------|--------|-----|
| WN Needle - WN SF | 0.6061 | 0.4692 | Inf | 1.2919 | 0.2357 | ns |
| MN SF - WN SF | 1.9395 | 0.4644 | Inf | 4.1764 | 0.0002 | *** |

Appendix Table 78
Pairwise comparison letter code

| Diet | Route | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|------|--------|--------|--------|-----|------------|------------|--------|
| WN | SF | 1.3643 | 0.3355 | Inf | 0.5262 | 2.2024 | a |
| WN | Needle | 1.9705 | 0.3279 | Inf | 1.1514 | 2.7895 | ab |
| MN | Needle | 2.9095 | 0.3223 | Inf | 2.1046 | 3.7144 | bc |
| MN | SF | 3.3039 | 0.3211 | Inf | 2.5019 | 4.1058 | c |

Panel e

Here, we investigated the lymph node barrier function in well-nourished and malnourished BALB/c mice infected with *Leishmania donovani* parasite via sand fly bites. The accumulation of intradermally injected 10,000 kDa-Dextran in the draining lymph nodes 72 h post sand fly bite were analyzed by Flow cytometry. We analyzed a total of $N=19$ BALB/c mice (MN_SF=9, WN_SF=10). For the data analysis, we tested Poisson and negative binomial regression models of the normalized cell counts, or beta regression after conversion of percentiles to ratios. Based on the Akaike information criterion (AIC), we selected a beta_regression model for the data analysis post data conversion to ratios. The model fit of the data was reasonable producing no statistically significant departure from 1 for its dispersion ratio (0.9284), but producing only a pseudo- R^2 of 0.1987. The model output showed that “Diet” was a statistically significant predictors (Appendix table 79) and its inclusion made the model distinct from the null model (Appendix table 80), showing that statistically significantly more Dextran was retained in draining lymph nodes from well-nourished BALB/c mice.

Appendix Table 79
Beta regression model output

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|-------------|----------|------------|---------|----------|------|
| (Intercept) | -1.2997 | 0.2663 | -4.8806 | <0.0001 | **** |
| DietWN | 0.7628 | 0.3418 | 2.2315 | 0.0256 | * |

Appendix Table 80
Predictor significance in the model

| Model | #Df | LogLik | Df | Chisq | Pr(>Chisq) | sig. |
|-------|-----|---------|----|-------|------------|------|
| Null | 3 | 10.1577 | NA | NA | NA | ? |

| | | | | | | |
|------|---|--------|----|--------|--------|---|
| Diet | 2 | 7.8903 | -1 | 4.5349 | 0.0332 | * |
|------|---|--------|----|--------|--------|---|

As the only predictor variable was dichotomous, there was strictly no need for a pairwise comparison. But we performed one anyway to ensure that the approach via estimated marginal means was comparable to the model output. The pairwise comparison based on the estimated marginal means showed that different nutritional statuses produced statistical significance comparable to the model output (Appendix table 81 & Appendix table 82). This supported the hypothesis that malnourishment resulted in a breakdown of the lymph node barrier, which could explained the increased parasite dissemination to the spleen in malnourished mice.

Appendix Table 81
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------|----------|--------|-----|---------|---------|------|
| MN - WN | -0.1547 | 0.0674 | Inf | -2.2944 | 0.0218 | * |

Appendix Table 82
Pairwise comparison letter code

| Diet | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|------|--------|--------|-----|------------|------------|--------|
| MN | 0.2142 | 0.0448 | Inf | 0.1137 | 0.3147 | a |
| WN | 0.3689 | 0.0516 | Inf | 0.2534 | 0.4845 | b |

Panel f

Whereas figure 2e looked at the retention of Dextran in draining lymph nodes, here, we investigated the accumulation of intradermally injected 10,000 kDa-Dextran in the spleen 72 h post sand fly bite, which required transition through the draining lymph node. The samples were also analyzed by Flow cytometry. We analyzed a total of $N=19$ BALB/c mice (MN_SF=9, WN_SF=10). This were the same mice as in figure 2e. For the data analysis, we tested Poisson and negative binomial regression models of the normalized cell counts, or beta regression after conversion of percentiles to ratios. Based on the Akaike information criterion (AIC), we selected a beta_regression model for the data analysis post data re-scaling. The model fit of the data was reasonable producing no statistically significant departure from 1 for its dispersion ratio (1.119), producing a pseudo- R^2 of 0.893. The model output showed that “Diet” was a statistically significant predictors (Appendix table 83) and its inclusion made the model distinct from the null model (Appendix table 84), showing that statistically significantly more Dextran accumulated in spleens from malnourished BALB/c mice.

Appendix Table 83
Beta regression model output

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|-------------|----------|------------|----------|----------|------|
| (Intercept) | -3.2827 | 0.0485 | -67.7093 | <0.0001 | **** |
| DietWN | -0.9373 | 0.0862 | -10.8747 | <0.0001 | **** |

Appendix Table 84
Predictor significance in the model

| Model | #Df | LogLik | Df | Chisq | Pr(>Chisq) | sig. |
|-------|-----|---------|----|---------|------------|------|
| Null | 3 | 78.0812 | NA | NA | NA | ? |
| Diet | 2 | 58.7547 | -1 | 38.6529 | <0.0001 | **** |

As the only predictor variable was dichotomous, there was strictly no need for a pairwise comparison. But we performed one anyway to ensure that the approach via estimated marginal means was comparable to the model output. The pairwise comparison based on the estimated marginal means showed that different nutritional statuses produced statistical significance comparable to the model output (Appendix table 85 & Appendix table 86). In agreement with the data from figure 2e, this data further supported the hypothesis that malnourishment resulted in a breakdown of the lymph node barrier, which could explained the increased parasite dissemination to the spleen in malnourished mice observed in figures 2c-d.

Appendix Table 85
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------|----------|-------|-----|---------|---------|------|
| MN - WN | 0.0217 | 0.002 | Inf | 11.0138 | <0.0001 | **** |

Appendix Table 86
Pairwise comparison letter code

| Diet | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|------|--------|--------|-----|------------|------------|--------|
| WN | 0.0145 | 0.0010 | Inf | 0.0122 | 0.0168 | a |
| MN | 0.0362 | 0.0017 | Inf | 0.0324 | 0.0400 | b |

Figure 3

Panel a

Data analysis

In figure 3a, we present the longitudinal weekly observation of mouse body weight pre and post *Leishmania donovani* infection, either by needle inoculation (needle), sand fly transmission (SF) or not at all (Naive). Information of a total of $N=45$ BALB/c mice (WN_Control: 8, MN_Control: 8, WN_Needle: 7, MN_Needle: 7, WN_SF: 8, MN_SF: 7) over the course of 22 weeks are shown here; “Week_0” being the weight before shipment, “Week_6” being the first week post-infection, and “Week_22” being the final week before the termination of the experiment.

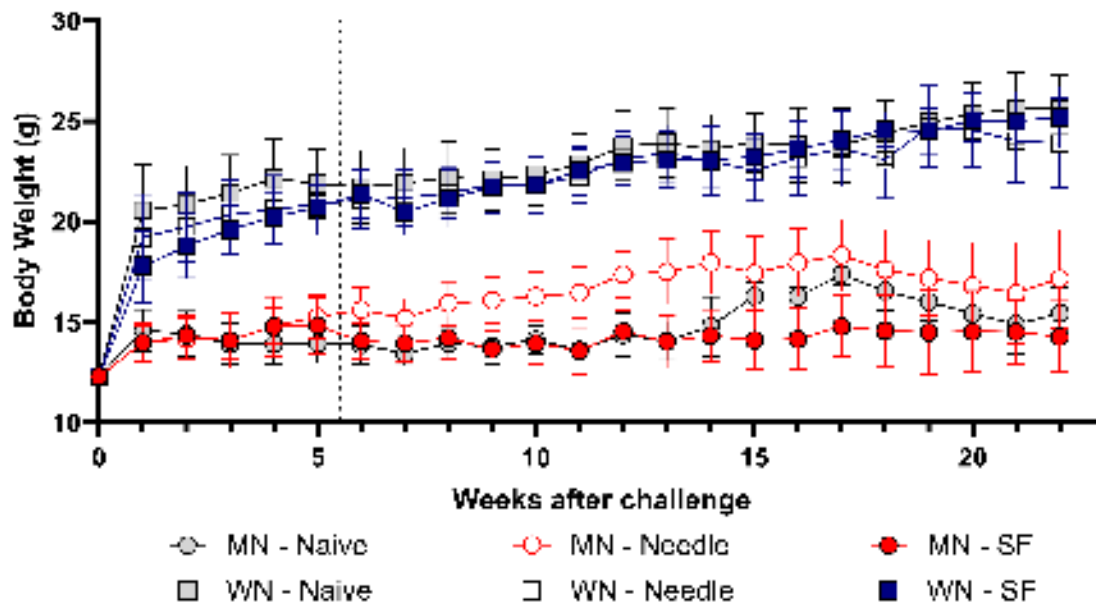


Fig.3a: This an extended version of the main figure 3a from the publication showing as well the pre-infection time-points. The dotted line marked the point of mouse infection.

We would need to analyze the data with a three-way mixed approach that included repeated measures to account for the three predictors; “Time_point” being the within-subject factor, while “Diet” and “Route” were the between-subject factors in the analysis with “Weight_g” being the dependent outcome variable.

For a three-way mixed ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance

- Homogeneity of Covariance
- No significant outliers
- Assumption of sphericity

Initial assumption assessment indicated that the Gaussian distribution assumption was not met along with the occurrence of several extreme outliers. Data transformation by Box-Cox power transformation reduced the magnitude of violation, although it did not completely remove it. Either way, we present the analysis of the assumption assessment with the transformed data below. Thus, data distribution and variance appear different in the main figure in the publication from the once that were used in the analysis post transformation.

Assumption analyses

Data normality

The assessment of the Box-Cox power transformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by all three predictors. Note that all groups consisted of N =WN_Control: 8, MN_Control: 8, WN_Needle: 7, MN_Needle: 7, WN_SF: 8, MN_SF: 7 individuals, which made groups too small to assess data distribution reliably by Shapiro-Wilks test. In spite of this, we performed the analyses by Shapiro-Wilks test (Appendix table 87) and QQ-pots (Fig.3a-1) and found deviations from normality.

Appendix Table 87
Univariate Shapiro-Wilks test results

| Diet | Route | Time_point | variable | statistic | p | Outcome |
|---------------|---------|------------|----------|-----------|--------|---------|
| Pre-Infection | | | | | | |
| WN | Control | Week_0 | Counts | 0.7729 | 0.0146 | sig. |
| MN | Control | Week_0 | Counts | 0.9118 | 0.3671 | ns |
| WN | Needle | Week_0 | Counts | 0.6644 | 0.0015 | sig. |
| MN | Needle | Week_0 | Counts | 0.8181 | 0.0615 | ns |
| WN | SF | Week_0 | Counts | 0.7823 | 0.0184 | sig. |
| MN | SF | Week_0 | Counts | 0.8333 | 0.0860 | ns |
| WN | Control | Week_1 | Counts | 0.8468 | 0.0884 | ns |
| MN | Control | Week_1 | Counts | 0.9006 | 0.2923 | ns |
| WN | Needle | Week_1 | Counts | 0.9335 | 0.5814 | ns |
| MN | Needle | Week_1 | Counts | 0.9593 | 0.8128 | ns |
| WN | SF | Week_1 | Counts | 0.8352 | 0.0673 | ns |
| MN | SF | Week_1 | Counts | 0.8678 | 0.1774 | ns |
| WN | Control | Week_2 | Counts | 0.8406 | 0.0764 | ns |
| MN | Control | Week_2 | Counts | 0.8894 | 0.2309 | ns |
| WN | Needle | Week_2 | Counts | 0.9363 | 0.6056 | ns |

| | | | | | | |
|----|---------|--------|--------|--------|--------|------|
| MN | Needle | Week_2 | Counts | 0.8935 | 0.2932 | ns |
| WN | SF | Week_2 | Counts | 0.8253 | 0.0531 | ns |
| MN | SF | Week_2 | Counts | 0.9515 | 0.7437 | ns |
| WN | Control | Week_3 | Counts | 0.8942 | 0.2558 | ns |
| MN | Control | Week_3 | Counts | 0.9273 | 0.4919 | ns |
| WN | Needle | Week_3 | Counts | 0.8753 | 0.2065 | ns |
| MN | Needle | Week_3 | Counts | 0.8073 | 0.0483 | sig. |
| WN | SF | Week_3 | Counts | 0.8960 | 0.2656 | ns |
| MN | SF | Week_3 | Counts | 0.9739 | 0.9249 | ns |
| WN | Control | Week_4 | Counts | 0.9525 | 0.7363 | ns |
| MN | Control | Week_4 | Counts | 0.9198 | 0.4281 | ns |
| WN | Needle | Week_4 | Counts | 0.9146 | 0.4285 | ns |
| MN | Needle | Week_4 | Counts | 0.8741 | 0.2016 | ns |
| WN | SF | Week_4 | Counts | 0.9291 | 0.5079 | ns |
| MN | SF | Week_4 | Counts | 0.9746 | 0.9292 | ns |
| WN | Control | Week_5 | Counts | 0.9659 | 0.8639 | ns |
| MN | Control | Week_5 | Counts | 0.8773 | 0.1773 | ns |
| WN | Needle | Week_5 | Counts | 0.9319 | 0.5674 | ns |
| MN | Needle | Week_5 | Counts | 0.8583 | 0.1463 | ns |
| WN | SF | Week_5 | Counts | 0.9314 | 0.5289 | ns |
| MN | SF | Week_5 | Counts | 0.9633 | 0.8463 | ns |

Post-Infestation

| | | | | | | |
|----|---------|--------|--------|--------|--------|----|
| WN | Control | Week_6 | Counts | 0.9440 | 0.6508 | ns |
| MN | Control | Week_6 | Counts | 0.9299 | 0.5147 | ns |
| WN | Needle | Week_6 | Counts | 0.9699 | 0.8976 | ns |
| MN | Needle | Week_6 | Counts | 0.8327 | 0.0848 | ns |
| WN | SF | Week_6 | Counts | 0.9879 | 0.9911 | ns |
| MN | SF | Week_6 | Counts | 0.9779 | 0.9487 | ns |
| WN | Control | Week_7 | Counts | 0.9637 | 0.8444 | ns |
| MN | Control | Week_7 | Counts | 0.9139 | 0.3825 | ns |
| WN | Needle | Week_7 | Counts | 0.9147 | 0.4296 | ns |
| MN | Needle | Week_7 | Counts | 0.9199 | 0.4688 | ns |
| WN | SF | Week_7 | Counts | 0.9801 | 0.9632 | ns |
| MN | SF | Week_7 | Counts | 0.9295 | 0.5467 | ns |
| WN | Control | Week_8 | Counts | 0.9699 | 0.8969 | ns |
| MN | Control | Week_8 | Counts | 0.9504 | 0.7148 | ns |
| WN | Needle | Week_8 | Counts | 0.9015 | 0.3402 | ns |
| MN | Needle | Week_8 | Counts | 0.8640 | 0.1643 | ns |
| WN | SF | Week_8 | Counts | 0.9431 | 0.6420 | ns |
| MN | SF | Week_8 | Counts | 0.8841 | 0.2453 | ns |

| | | | | | | |
|----|---------|---------|--------|--------|--------|------|
| WN | Control | Week_9 | Counts | 0.9563 | 0.7743 | ns |
| MN | Control | Week_9 | Counts | 0.9378 | 0.5895 | ns |
| WN | Needle | Week_9 | Counts | 0.8770 | 0.2135 | ns |
| MN | Needle | Week_9 | Counts | 0.9078 | 0.3810 | ns |
| WN | SF | Week_9 | Counts | 0.9499 | 0.7105 | ns |
| MN | SF | Week_9 | Counts | 0.9522 | 0.7493 | ns |
| WN | Control | Week_10 | Counts | 0.9366 | 0.5777 | ns |
| MN | Control | Week_10 | Counts | 0.9605 | 0.8145 | ns |
| WN | Needle | Week_10 | Counts | 0.9026 | 0.3472 | ns |
| MN | Needle | Week_10 | Counts | 0.9179 | 0.4533 | ns |
| WN | SF | Week_10 | Counts | 0.9133 | 0.3782 | ns |
| MN | SF | Week_10 | Counts | 0.9534 | 0.7606 | ns |
| WN | Control | Week_11 | Counts | 0.9437 | 0.6478 | ns |
| MN | Control | Week_11 | Counts | 0.9641 | 0.8477 | ns |
| WN | Needle | Week_11 | Counts | 0.9074 | 0.3782 | ns |
| MN | Needle | Week_11 | Counts | 0.8722 | 0.1940 | ns |
| WN | SF | Week_11 | Counts | 0.9294 | 0.5107 | ns |
| MN | SF | Week_11 | Counts | 0.9553 | 0.7771 | ns |
| WN | Control | Week_12 | Counts | 0.9587 | 0.7981 | ns |
| MN | Control | Week_12 | Counts | 0.9428 | 0.6389 | ns |
| WN | Needle | Week_12 | Counts | 0.9434 | 0.6696 | ns |
| MN | Needle | Week_12 | Counts | 0.9293 | 0.5452 | ns |
| WN | SF | Week_12 | Counts | 0.9507 | 0.7186 | ns |
| MN | SF | Week_12 | Counts | 0.9283 | 0.5365 | ns |
| WN | Control | Week_13 | Counts | 0.9768 | 0.9453 | ns |
| MN | Control | Week_13 | Counts | 0.9050 | 0.3205 | ns |
| WN | Needle | Week_13 | Counts | 0.9768 | 0.9426 | ns |
| MN | Needle | Week_13 | Counts | 0.9225 | 0.4887 | ns |
| WN | SF | Week_13 | Counts | 0.9049 | 0.3195 | ns |
| MN | SF | Week_13 | Counts | 0.9002 | 0.3323 | ns |
| WN | Control | Week_14 | Counts | 0.9521 | 0.7323 | ns |
| MN | Control | Week_14 | Counts | 0.8924 | 0.2464 | ns |
| WN | Needle | Week_14 | Counts | 0.9310 | 0.5592 | ns |
| MN | Needle | Week_14 | Counts | 0.9140 | 0.4244 | ns |
| WN | SF | Week_14 | Counts | 0.8788 | 0.1834 | ns |
| MN | SF | Week_14 | Counts | 0.9498 | 0.7279 | ns |
| WN | Control | Week_15 | Counts | 0.9124 | 0.3715 | ns |
| MN | Control | Week_15 | Counts | 0.8167 | 0.0431 | sig. |
| WN | Needle | Week_15 | Counts | 0.7351 | 0.0088 | sig. |
| MN | Needle | Week_15 | Counts | 0.9505 | 0.7338 | ns |
| WN | SF | Week_15 | Counts | 0.9126 | 0.3729 | ns |

| | | | | | | |
|----|---------|---------|--------|--------|--------|------|
| MN | SF | Week_15 | Counts | 0.9603 | 0.8210 | ns |
| WN | Control | Week_16 | Counts | 0.8604 | 0.1212 | ns |
| MN | Control | Week_16 | Counts | 0.8289 | 0.0578 | ns |
| WN | Needle | Week_16 | Counts | 0.7123 | 0.0050 | sig. |
| MN | Needle | Week_16 | Counts | 0.9455 | 0.6885 | ns |
| WN | SF | Week_16 | Counts | 0.8795 | 0.1861 | ns |
| MN | SF | Week_16 | Counts | 0.9444 | 0.6783 | ns |
| WN | Control | Week_17 | Counts | 0.8210 | 0.0478 | sig. |
| MN | Control | Week_17 | Counts | 0.8694 | 0.1487 | ns |
| WN | Needle | Week_17 | Counts | 0.8980 | 0.3190 | ns |
| MN | Needle | Week_17 | Counts | 0.9786 | 0.9522 | ns |
| WN | SF | Week_17 | Counts | 0.8581 | 0.1149 | ns |
| MN | SF | Week_17 | Counts | 0.9189 | 0.4612 | ns |
| WN | Control | Week_18 | Counts | 0.8087 | 0.0354 | sig. |
| MN | Control | Week_18 | Counts | 0.9667 | 0.8713 | ns |
| WN | Needle | Week_18 | Counts | 0.8892 | 0.2706 | ns |
| MN | Needle | Week_18 | Counts | 0.9528 | 0.7550 | ns |
| WN | SF | Week_18 | Counts | 0.8360 | 0.0686 | ns |
| MN | SF | Week_18 | Counts | 0.9453 | 0.6869 | ns |
| WN | Control | Week_19 | Counts | 0.8965 | 0.2689 | ns |
| MN | Control | Week_19 | Counts | 0.9282 | 0.4996 | ns |
| WN | Needle | Week_19 | Counts | 0.6529 | 0.0011 | sig. |
| MN | Needle | Week_19 | Counts | 0.9179 | 0.4532 | ns |
| WN | SF | Week_19 | Counts | 0.8780 | 0.1801 | ns |
| MN | SF | Week_19 | Counts | 0.9285 | 0.5383 | ns |
| WN | Control | Week_20 | Counts | 0.8508 | 0.0971 | ns |
| MN | Control | Week_20 | Counts | 0.9540 | 0.7517 | ns |
| WN | Needle | Week_20 | Counts | 0.7695 | 0.0202 | sig. |
| MN | Needle | Week_20 | Counts | 0.9215 | 0.4808 | ns |
| WN | SF | Week_20 | Counts | 0.8922 | 0.2453 | ns |
| MN | SF | Week_20 | Counts | 0.9223 | 0.4875 | ns |
| WN | Control | Week_21 | Counts | 0.9517 | 0.7285 | ns |
| MN | Control | Week_21 | Counts | 0.9221 | 0.4467 | ns |
| WN | Needle | Week_21 | Counts | 0.8350 | 0.0893 | ns |
| MN | Needle | Week_21 | Counts | 0.9039 | 0.3553 | ns |
| WN | SF | Week_21 | Counts | 0.8391 | 0.0737 | ns |
| MN | SF | Week_21 | Counts | 0.9255 | 0.5133 | ns |
| WN | Control | Week_22 | Counts | 0.9688 | 0.8884 | ns |
| MN | Control | Week_22 | Counts | 0.9392 | 0.6311 | ns |
| WN | Needle | Week_22 | Counts | 0.8309 | 0.0816 | ns |
| MN | Needle | Week_22 | Counts | 0.9453 | 0.6865 | ns |

| | | | | | | |
|----|----|---------|--------|--------|--------|----|
| WN | SF | Week_22 | Counts | 0.8552 | 0.1075 | ns |
| MN | SF | Week_22 | Counts | 0.9137 | 0.4219 | ns |



Fig.3a-1: QQ-plots of repeatedly measured mouse weights split into groups by predictor variables

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset split by the within-subject factor ("Time_point"). The analysis output showed that assumption of homogeneity between groups held for each week (Appendix table 88).

Appendix Table 88
Assessment of homogeneity of variance by week

| Weeks p.i. | df1 | df2 | statistic | p | sig. |
|------------------|-----|-----|-----------|--------|------|
| Pre-Infection | | | | | |
| Week_0 | 5 | 39 | 0.5518 | 0.7360 | ns |
| Week_1 | 5 | 39 | 1.2268 | 0.3151 | ns |
| Week_2 | 5 | 39 | 0.4165 | 0.8344 | ns |
| Week_3 | 5 | 39 | 0.3987 | 0.8467 | ns |
| Week_4 | 5 | 39 | 0.8811 | 0.5029 | ns |
| Week_5 | 5 | 39 | 0.6984 | 0.6279 | ns |
| Post-Infestation | | | | | |
| Week_6 | 5 | 39 | 1.0550 | 0.3998 | ns |
| Week_7 | 5 | 39 | 1.1844 | 0.3344 | ns |
| Week_8 | 5 | 39 | 0.8324 | 0.5347 | ns |
| Week_9 | 5 | 39 | 0.4061 | 0.8416 | ns |
| Week_10 | 5 | 39 | 0.5260 | 0.7551 | ns |
| Week_11 | 5 | 39 | 0.3293 | 0.8922 | ns |
| Week_12 | 5 | 39 | 0.5888 | 0.7084 | ns |
| Week_13 | 5 | 39 | 0.2664 | 0.9287 | ns |
| Week_14 | 5 | 39 | 0.1285 | 0.9850 | ns |
| Week_15 | 5 | 39 | 0.8254 | 0.5393 | ns |
| Week_16 | 5 | 39 | 1.5470 | 0.1979 | ns |
| Week_17 | 5 | 39 | 1.6538 | 0.1688 | ns |
| Week_18 | 5 | 39 | 0.9135 | 0.4824 | ns |
| Week_19 | 5 | 39 | 0.7931 | 0.5612 | ns |
| Week_20 | 5 | 39 | 0.9508 | 0.4595 | ns |
| Week_21 | 5 | 39 | 0.2343 | 0.9451 | ns |
| Week_22 | 5 | 38 | 0.3294 | 0.8921 | ns |

Outliers

It can be difficult to determine outliers in small datasets reliably as the analysis is dependent on the interquartile range of the data per group. We attempted it anyway and found a total of 53 hypothetical outliers of which 7 were classed as extreme (Appendix table 89).

Appendix Table 89
List of possible outliers

| Diet | Route | Time_point | is.outlier | is.extreme |
|------------------|---------|------------|------------|------------|
| Pre-Infection | | | | |
| WN | Control | Week_0 | TRUE | FALSE |
| WN | Control | Week_1 | TRUE | FALSE |
| MN | Control | Week_1 | TRUE | FALSE |
| MN | Control | Week_1 | TRUE | TRUE |
| MN | Needle | Week_1 | TRUE | FALSE |
| WN | SF | Week_1 | TRUE | FALSE |
| MN | Control | Week_2 | TRUE | FALSE |
| MN | Control | Week_2 | TRUE | TRUE |
| WN | SF | Week_2 | TRUE | FALSE |
| WN | SF | Week_2 | TRUE | FALSE |
| MN | SF | Week_2 | TRUE | FALSE |
| WN | Control | Week_3 | TRUE | FALSE |
| MN | Control | Week_3 | TRUE | FALSE |
| MN | Control | Week_3 | TRUE | FALSE |
| MN | Needle | Week_3 | TRUE | FALSE |
| WN | SF | Week_3 | TRUE | FALSE |
| MN | SF | Week_3 | TRUE | FALSE |
| MN | Control | Week_4 | TRUE | FALSE |
| MN | Control | Week_4 | TRUE | FALSE |
| MN | Needle | Week_4 | TRUE | TRUE |
| WN | SF | Week_4 | TRUE | FALSE |
| MN | Control | Week_5 | TRUE | TRUE |
| MN | Control | Week_5 | TRUE | FALSE |
| WN | SF | Week_5 | TRUE | FALSE |
| WN | SF | Week_5 | TRUE | FALSE |
| Post-Infestation | | | | |
| MN | Control | Week_6 | TRUE | FALSE |
| MN | Control | Week_6 | TRUE | FALSE |
| WN | SF | Week_9 | TRUE | FALSE |
| WN | Needle | Week_10 | TRUE | FALSE |
| WN | Needle | Week_11 | TRUE | FALSE |
| WN | SF | Week_11 | TRUE | FALSE |
| WN | Needle | Week_12 | TRUE | FALSE |
| WN | Needle | Week_14 | TRUE | FALSE |
| MN | Control | Week_15 | TRUE | FALSE |

| | | | | |
|----|---------|---------|------|-------|
| WN | Needle | Week_15 | TRUE | FALSE |
| WN | SF | Week_15 | TRUE | FALSE |
| MN | SF | Week_15 | TRUE | FALSE |
| MN | Control | Week_16 | TRUE | FALSE |
| WN | Needle | Week_16 | TRUE | TRUE |
| MN | Control | Week_17 | TRUE | FALSE |
| WN | Needle | Week_17 | TRUE | FALSE |
| MN | Control | Week_18 | TRUE | FALSE |
| WN | Needle | Week_18 | TRUE | FALSE |
| WN | Needle | Week_19 | TRUE | TRUE |
| MN | Needle | Week_19 | TRUE | FALSE |
| WN | Needle | Week_20 | TRUE | TRUE |
| MN | Needle | Week_20 | TRUE | FALSE |
| WN | Needle | Week_21 | TRUE | FALSE |
| MN | Needle | Week_21 | TRUE | FALSE |
| MN | Control | Week_22 | TRUE | FALSE |
| MN | Control | Week_22 | TRUE | FALSE |
| WN | Needle | Week_22 | TRUE | FALSE |
| MN | Needle | Week_22 | TRUE | FALSE |

Three-way mixed analysis

Based on the assumption tests, we decided to apply a Robust three-way ANOVA to the dataset to determine the effects of “Diet”, infection “Route” and time pre and post infection (“Time_point”) on mouse weight over time (Appendix table 90). The test output showed that all three individual predictors were statistically significant, so were all two-way and the three-way interaction terms.

Appendix Table 90
Robust three-way mixed ANOVA

| Predictors | value | p.value | sig. |
|-----------------------|------------|---------|------|
| Diet | 4726.0759 | 0.0001 | **** |
| Route | 53.7814 | 0.0001 | **** |
| Time_point | 15104.9064 | 0.0010 | *** |
| Diet:Route | 155.4204 | 0.0010 | *** |
| Diet:Time_point | 4280.0234 | 0.0010 | *** |
| Route:Time_point | 106.9048 | 0.0010 | *** |
| Diet:Route:Time_point | 204.8501 | 0.0010 | *** |

We looked for main effects by splitting the data by the within-subject factor (“Time_point”) and analyzed the remaining two predictor (“Diet” and “Route”) by a Robust two-way ANOVA. The results showed that

both predictors, “Diet” and “Route”, produced statistically significant p-values. While “Diet” had always statistical significance with the exception of “Week_0”, which was unsurprising considering the large gap in body weight between well-nourished and malnourished mice otherwise observed during the 22 week period (Fig. 3a). “Route” was only a statistically significant predictor between “Week_9” and “Week_17”, and the interaction term was statistically significant between “Week_7” and “Week_18” (Appendix table 91). This suggested that the effects of the “Route” of infection were only observed for a limited period of time post infection, while the effects of “Diet” were omnipresent and already established at the point of infection. The interaction suggested that within one or both dietary groups statistically significant differences were observed due to infection route.

Appendix Table 91
Robust two-way ANOVA

| Weeks p.i. | Predictor | value | p.value | Sig. |
|------------------|------------|----------|---------|------|
| Pre-Infection | | | | |
| Week_0 | Diet | 0.6436 | 0.429 | ns |
| Week_0 | Route | 4.8913 | 0.116 | ns |
| Week_0 | Diet:Route | 0.5024 | 0.786 | ns |
| Week_1 | Diet | 83.2007 | 0.001 | *** |
| Week_1 | Route | 5.6282 | 0.088 | + |
| Week_1 | Diet:Route | 2.2941 | 0.349 | ns |
| Week_2 | Diet | 128.0593 | 0.001 | *** |
| Week_2 | Route | 2.6197 | 0.298 | ns |
| Week_2 | Diet:Route | 2.3070 | 0.342 | ns |
| Week_3 | Diet | 187.7605 | 0.001 | *** |
| Week_3 | Route | 1.5396 | 0.484 | ns |
| Week_3 | Diet:Route | 2.3585 | 0.335 | ns |
| Week_4 | Diet | 192.4720 | 0.001 | *** |
| Week_4 | Route | 0.3976 | 0.826 | ns |
| Week_4 | Diet:Route | 6.0356 | 0.073 | + |
| Week_5 | Diet | 212.5551 | 0.001 | *** |
| Week_5 | Route | 0.4535 | 0.804 | ns |
| Week_5 | Diet:Route | 5.4285 | 0.091 | + |
| Post-Infestation | | | | |
| Week_6 | Diet | 262.5681 | 0.001 | *** |
| Week_6 | Route | 2.5042 | 0.313 | ns |
| Week_6 | Diet:Route | 6.2856 | 0.064 | + |
| Week_7 | Diet | 330.1662 | 0.001 | *** |
| Week_7 | Route | 5.1005 | 0.106 | ns |
| Week_7 | Diet:Route | 7.2688 | 0.045 | * |

| | | | | |
|---------|------------|----------|-------|-----|
| Week_8 | Diet | 263.7557 | 0.001 | *** |
| Week_8 | Route | 4.9514 | 0.110 | ns |
| Week_8 | Diet:Route | 7.9498 | 0.034 | * |
| Week_9 | Diet | 335.2713 | 0.001 | *** |
| Week_9 | Route | 8.9642 | 0.023 | * |
| Week_9 | Diet:Route | 9.4158 | 0.019 | * |
| Week_10 | Diet | 304.2046 | 0.001 | *** |
| Week_10 | Route | 6.7550 | 0.053 | + |
| Week_10 | Diet:Route | 9.4577 | 0.019 | * |
| Week_11 | Diet | 378.3575 | 0.001 | *** |
| Week_11 | Route | 10.0779 | 0.016 | * |
| Week_11 | Diet:Route | 16.3481 | 0.002 | ** |
| Week_12 | Diet | 326.4604 | 0.001 | *** |
| Week_12 | Route | 12.5730 | 0.006 | ** |
| Week_12 | Diet:Route | 14.7796 | 0.003 | ** |
| Week_13 | Diet | 302.1738 | 0.001 | *** |
| Week_13 | Route | 13.4748 | 0.005 | ** |
| Week_13 | Diet:Route | 14.6520 | 0.004 | ** |
| Week_14 | Diet | 208.9694 | 0.001 | *** |
| Week_14 | Route | 10.6331 | 0.013 | * |
| Week_14 | Diet:Route | 13.2573 | 0.005 | ** |
| Week_15 | Diet | 231.8533 | 0.001 | *** |
| Week_15 | Route | 10.0367 | 0.017 | * |
| Week_15 | Diet:Route | 11.3226 | 0.011 | * |
| Week_16 | Diet | 210.5225 | 0.001 | *** |
| Week_16 | Route | 8.7925 | 0.026 | * |
| Week_16 | Diet:Route | 11.9954 | 0.009 | ** |
| Week_17 | Diet | 177.0197 | 0.001 | *** |
| Week_17 | Route | 7.6225 | 0.040 | * |
| Week_17 | Diet:Route | 11.2424 | 0.011 | * |
| Week_18 | Diet | 208.5641 | 0.001 | *** |
| Week_18 | Route | 3.5529 | 0.204 | ns |
| Week_18 | Diet:Route | 11.4103 | 0.011 | * |
| Week_19 | Diet | 230.7852 | 0.001 | *** |
| Week_19 | Route | 4.5003 | 0.139 | ns |
| Week_19 | Diet:Route | 3.4689 | 0.212 | ns |
| Week_20 | Diet | 259.4895 | 0.001 | *** |
| Week_20 | Route | 2.0155 | 0.395 | ns |
| Week_20 | Diet:Route | 4.1171 | 0.163 | ns |
| Week_21 | Diet | 227.8845 | 0.001 | *** |
| Week_21 | Route | 0.7795 | 0.689 | ns |

| | | | | |
|---------|------------|----------|-------|-----|
| Week_21 | Diet:Route | 4.3443 | 0.143 | ns |
| Week_22 | Diet | 215.7785 | 0.001 | *** |
| Week_22 | Route | 2.1574 | 0.367 | ns |
| Week_22 | Diet:Route | 7.1401 | 0.048 | * |

For the analysis of the simple main effect for each respective between-subject factor, we performed Robust one-way ANOVAs with individual between-subject factor of the data split by the other two predictors. The results showed that “Diet” caused statistically significant differences with the exception of “Week_0”, which was prior to the assignment of special diets (Appendix table 92). “Route only showed occasionally statistical significant difference; most commonly between”Week_6” and “Week_18”, which was only associated with the malnourished group (Appendix table 93).

Appendix Table 92
Robust one-way ANOVA

| Time_point | Factor | Effect | test | df1 | df2 | p.value | effsize | CI_lower | CI_upper | Sig. |
|----------------|---------|--------|----------|-----|---------|---------|---------|----------|----------|------|
| Split by Route | | | | | | | | | | |
| Week_0 | Control | Diet | 0.9463 | 1 | 13.9443 | 0.3472 | 0.3265 | 0.0009 | 0.7239 | ns |
| Week_0 | Needle | Diet | 0.0742 | 1 | 8.1104 | 0.7921 | 0.0880 | 0.0018 | 0.8640 | ns |
| Week_0 | SF | Diet | 0.0073 | 1 | 12.9968 | 0.9334 | 0.2208 | 0.0000 | 0.6874 | ns |
| Week_1 | Control | Diet | 30.9197 | 1 | 10.5864 | 0.0002 | 0.9867 | 0.7579 | 1.1607 | *** |
| Week_1 | Needle | Diet | 33.6082 | 1 | 8.7119 | 0.0003 | 1.0360 | 0.9235 | 1.1585 | *** |
| Week_1 | SF | Diet | 19.4931 | 1 | 10.7930 | 0.0011 | 0.9022 | 0.5425 | 1.0997 | ** |
| Week_2 | Control | Diet | 46.6180 | 1 | 12.5452 | <0.0001 | 1.0575 | 0.9074 | 1.1649 | **** |
| Week_2 | Needle | Diet | 49.7907 | 1 | 10.7859 | <0.0001 | 1.0994 | 0.9863 | 1.2523 | **** |
| Week_2 | SF | Diet | 32.7851 | 1 | 11.8409 | 0.0001 | 0.9999 | 0.8331 | 1.1639 | *** |
| Week_3 | Control | Diet | 71.4565 | 1 | 12.4770 | <0.0001 | 1.0818 | 0.9760 | 1.1524 | **** |
| Week_3 | Needle | Diet | 66.2027 | 1 | 10.2527 | <0.0001 | 1.1338 | 1.0156 | 1.2552 | **** |
| Week_3 | SF | Diet | 50.8531 | 1 | 11.8824 | <0.0001 | 1.0649 | 0.9792 | 1.1777 | **** |
| Week_4 | Control | Diet | 87.8417 | 1 | 12.1493 | <0.0001 | 1.1032 | 1.0040 | 1.1413 | **** |
| Week_4 | Needle | Diet | 60.9978 | 1 | 10.2379 | <0.0001 | 1.1344 | 1.0132 | 1.2331 | **** |
| Week_4 | SF | Diet | 45.9854 | 1 | 11.0139 | <0.0001 | 1.0600 | 0.9189 | 1.1884 | **** |
| Week_5 | Control | Diet | 100.3686 | 1 | 12.7279 | <0.0001 | 1.1053 | 1.0363 | 1.1542 | **** |
| Week_5 | Needle | Diet | 56.4055 | 1 | 11.6501 | <0.0001 | 1.0898 | 0.9942 | 1.2567 | **** |
| Week_5 | SF | Diet | 58.6004 | 1 | 10.7527 | <0.0001 | 1.0839 | 0.9806 | 1.2003 | **** |
| Week_6 | Control | Diet | 88.8819 | 1 | 12.0825 | <0.0001 | 1.0922 | 1.0234 | 1.1534 | **** |
| Week_6 | Needle | Diet | 54.4571 | 1 | 11.9346 | <0.0001 | 1.0689 | 0.9799 | 1.2291 | **** |
| Week_6 | SF | Diet | 138.7395 | 1 | 12.9947 | <0.0001 | 1.1099 | 1.0636 | 1.1608 | **** |
| Week_7 | Control | Diet | 139.0888 | 1 | 9.6090 | <0.0001 | 1.1113 | 1.0622 | 1.1520 | **** |
| Week_7 | Needle | Diet | 79.5424 | 1 | 11.6833 | <0.0001 | 1.1279 | 1.0372 | 1.2009 | **** |

| | | | | | | | | | | |
|---------|---------|------|----------|---|---------|---------|--------|--------|--------|------|
| Week_7 | SF | Diet | 115.0358 | 1 | 12.9933 | <0.0001 | 1.1055 | 1.0434 | 1.1664 | **** |
| Week_8 | Control | Diet | 108.6133 | 1 | 11.1301 | <0.0001 | 1.1069 | 1.0373 | 1.1390 | **** |
| Week_8 | Needle | Diet | 64.9819 | 1 | 11.9851 | <0.0001 | 1.1008 | 1.0198 | 1.1840 | **** |
| Week_8 | SF | Diet | 90.4757 | 1 | 12.9631 | <0.0001 | 1.0930 | 1.0282 | 1.1714 | **** |
| Week_9 | Control | Diet | 138.2405 | 1 | 12.6407 | <0.0001 | 1.0938 | 1.0567 | 1.1317 | **** |
| Week_9 | Needle | Diet | 59.2647 | 1 | 11.9764 | <0.0001 | 1.1281 | 1.0054 | 1.2280 | **** |
| Week_9 | SF | Diet | 156.4098 | 1 | 12.9750 | <0.0001 | 1.1141 | 1.0647 | 1.1674 | **** |
| Week_10 | Control | Diet | 142.1475 | 1 | 11.3023 | <0.0001 | 1.1193 | 1.0511 | 1.1490 | **** |
| Week_10 | Needle | Diet | 56.2825 | 1 | 11.9764 | <0.0001 | 1.1457 | 1.0089 | 1.2562 | **** |
| Week_10 | SF | Diet | 118.0082 | 1 | 12.9925 | <0.0001 | 1.0907 | 1.0405 | 1.1480 | **** |
| Week_11 | Control | Diet | 184.1228 | 1 | 12.2479 | <0.0001 | 1.0992 | 1.0666 | 1.1448 | **** |
| Week_11 | Needle | Diet | 59.3268 | 1 | 11.7074 | <0.0001 | 1.1027 | 1.0133 | 1.2393 | **** |
| Week_11 | SF | Diet | 158.6371 | 1 | 12.1492 | <0.0001 | 1.1072 | 1.0679 | 1.1475 | **** |
| Week_12 | Control | Diet | 124.1778 | 1 | 13.5899 | <0.0001 | 1.1083 | 1.0484 | 1.1454 | **** |
| Week_12 | Needle | Diet | 63.5146 | 1 | 11.9642 | <0.0001 | 1.1025 | 1.0221 | 1.2193 | **** |
| Week_12 | SF | Diet | 148.2799 | 1 | 12.9212 | <0.0001 | 1.1048 | 1.0781 | 1.1607 | **** |
| Week_13 | Control | Diet | 159.1247 | 1 | 12.9562 | <0.0001 | 1.1146 | 1.0659 | 1.1539 | **** |
| Week_13 | Needle | Diet | 43.9328 | 1 | 11.0735 | <0.0001 | 1.0647 | 0.9797 | 1.2014 | **** |
| Week_13 | SF | Diet | 124.1739 | 1 | 12.1224 | <0.0001 | 1.0937 | 1.0459 | 1.1608 | **** |
| Week_14 | Control | Diet | 81.6612 | 1 | 13.7559 | <0.0001 | 1.1121 | 1.0289 | 1.1862 | **** |
| Week_14 | Needle | Diet | 28.2635 | 1 | 11.9462 | 0.0002 | 1.1630 | 0.9098 | 1.2623 | *** |
| Week_14 | SF | Diet | 121.5173 | 1 | 12.1033 | <0.0001 | 1.0929 | 1.0455 | 1.1470 | **** |
| Week_15 | Control | Diet | 132.8303 | 1 | 11.5452 | <0.0001 | 1.1446 | 1.0555 | 1.1641 | **** |
| Week_15 | Needle | Diet | 26.5980 | 1 | 10.6701 | 0.0003 | 1.1250 | 0.8928 | 1.3944 | *** |
| Week_15 | SF | Diet | 127.4723 | 1 | 10.3884 | <0.0001 | 1.1094 | 1.0361 | 1.1579 | **** |
| Week_16 | Control | Diet | 100.3834 | 1 | 8.4872 | <0.0001 | 1.0950 | 1.0457 | 1.1333 | **** |
| Week_16 | Needle | Diet | 27.3372 | 1 | 11.9350 | 0.0002 | 1.1261 | 0.9201 | 1.2388 | *** |
| Week_16 | SF | Diet | 110.7167 | 1 | 11.2457 | <0.0001 | 1.0967 | 1.0502 | 1.1709 | **** |
| Week_17 | Control | Diet | 69.5823 | 1 | 8.7894 | <0.0001 | 1.0702 | 1.0164 | 1.1259 | **** |
| Week_17 | Needle | Diet | 25.4983 | 1 | 11.8638 | 0.0003 | 1.0843 | 0.8552 | 1.2132 | *** |
| Week_17 | SF | Diet | 104.7430 | 1 | 11.4557 | <0.0001 | 1.0962 | 1.0511 | 1.1639 | **** |
| Week_18 | Control | Diet | 108.3643 | 1 | 12.9680 | <0.0001 | 1.0997 | 1.0550 | 1.1702 | **** |
| Week_18 | Needle | Diet | 23.9856 | 1 | 11.6560 | 0.0004 | 1.0855 | 0.8390 | 1.2260 | *** |
| Week_18 | SF | Diet | 123.5300 | 1 | 7.7478 | <0.0001 | 1.0956 | 1.0486 | 1.1685 | **** |
| Week_19 | Control | Diet | 133.9525 | 1 | 12.8372 | <0.0001 | 1.0831 | 1.0591 | 1.1506 | **** |
| Week_19 | Needle | Diet | 46.3485 | 1 | 11.7773 | <0.0001 | 1.1568 | 0.9677 | 1.2498 | **** |
| Week_19 | SF | Diet | 82.2076 | 1 | 7.9091 | <0.0001 | 1.0804 | 1.0251 | 1.1832 | **** |
| Week_20 | Control | Diet | 157.5335 | 1 | 13.9259 | <0.0001 | 1.0929 | 1.0730 | 1.1631 | **** |
| Week_20 | Needle | Diet | 44.7878 | 1 | 10.7340 | <0.0001 | 1.1444 | 0.9522 | 1.2082 | **** |
| Week_20 | SF | Diet | 99.9882 | 1 | 7.8372 | <0.0001 | 1.0933 | 1.0481 | 1.1832 | **** |
| Week_21 | Control | Diet | 113.5715 | 1 | 13.8178 | <0.0001 | 1.0896 | 1.0582 | 1.1729 | **** |

| | | | | | | | | | | |
|---------|---------|------|----------|---|---------|---------|--------|--------|--------|------|
| Week_21 | Needle | Diet | 33.1991 | 1 | 10.5972 | 0.0001 | 1.0890 | 0.8607 | 1.2207 | *** |
| Week_21 | SF | Diet | 121.3469 | 1 | 11.0681 | <0.0001 | 1.1009 | 1.0605 | 1.1678 | **** |
| Week_22 | Control | Diet | 136.2265 | 1 | 12.9780 | <0.0001 | 1.1126 | 1.0586 | 1.1811 | **** |
| Week_22 | Needle | Diet | 25.6731 | 1 | 11.1438 | 0.0003 | 1.0823 | 0.7903 | 1.2356 | *** |
| Week_22 | SF | Diet | 111.3334 | 1 | 10.6612 | <0.0001 | 1.0940 | 1.0386 | 1.1744 | **** |

Split by Diet

| | | | | | | | | | | |
|---------|----|-------|---------|---|---------|--------|--------|--------|--------|-----|
| Week_0 | WN | Route | 1.9588 | 2 | 12.8725 | 0.1808 | 0.4893 | 0.1490 | 0.8093 | ns |
| Week_0 | MN | Route | 1.0762 | 2 | 12.1312 | 0.3713 | 0.4805 | 0.1011 | 0.8476 | ns |
| Week_1 | WN | Route | 2.2567 | 2 | 13.2118 | 0.1435 | 0.5778 | 0.2353 | 0.9016 | ns |
| Week_1 | MN | Route | 0.6409 | 2 | 12.6553 | 0.5431 | 0.4520 | 0.0778 | 0.9168 | ns |
| Week_2 | WN | Route | 1.6924 | 2 | 13.2826 | 0.2215 | 0.5245 | 0.1950 | 0.8207 | ns |
| Week_2 | MN | Route | 0.0597 | 2 | 12.6611 | 0.9423 | 0.3626 | 0.0496 | 0.9067 | ns |
| Week_3 | WN | Route | 1.6823 | 2 | 12.8046 | 0.2245 | 0.5462 | 0.1318 | 0.8698 | ns |
| Week_3 | MN | Route | 0.0564 | 2 | 12.3027 | 0.9454 | 0.3494 | 0.0647 | 0.9018 | ns |
| Week_4 | WN | Route | 1.8918 | 2 | 12.7117 | 0.1909 | 0.5329 | 0.1169 | 0.8900 | ns |
| Week_4 | MN | Route | 1.3314 | 2 | 12.1316 | 0.3001 | 0.5199 | 0.1351 | 0.9043 | ns |
| Week_5 | WN | Route | 0.8912 | 2 | 12.7927 | 0.4342 | 0.4278 | 0.0860 | 0.8367 | ns |
| Week_5 | MN | Route | 2.4199 | 2 | 12.2564 | 0.1301 | 0.5479 | 0.1046 | 0.8873 | ns |
| Week_6 | WN | Route | 0.2057 | 2 | 12.9847 | 0.8167 | 0.3726 | 0.0547 | 0.7028 | ns |
| Week_6 | MN | Route | 4.6012 | 2 | 12.5486 | 0.0317 | 0.7071 | 0.3539 | 0.9990 | * |
| Week_7 | WN | Route | 1.3374 | 2 | 13.0307 | 0.2963 | 0.4659 | 0.0746 | 0.8409 | ns |
| Week_7 | MN | Route | 6.6197 | 2 | 11.8250 | 0.0118 | 0.7229 | 0.4533 | 0.9427 | * |
| Week_8 | WN | Route | 0.5185 | 2 | 13.2073 | 0.6070 | 0.3676 | 0.0697 | 0.7614 | ns |
| Week_8 | MN | Route | 6.4573 | 2 | 12.2947 | 0.0121 | 0.7206 | 0.5076 | 0.9312 | * |
| Week_9 | WN | Route | 0.0958 | 2 | 13.2133 | 0.9093 | 0.3300 | 0.0387 | 0.7494 | ns |
| Week_9 | MN | Route | 9.4920 | 2 | 12.4183 | 0.0032 | 0.7882 | 0.6314 | 0.9290 | ** |
| Week_10 | WN | Route | 0.2026 | 2 | 13.3101 | 0.8191 | 0.3207 | 0.0838 | 0.7937 | ns |
| Week_10 | MN | Route | 7.8905 | 2 | 11.8254 | 0.0067 | 0.7947 | 0.5708 | 0.9749 | ** |
| Week_11 | WN | Route | 0.2315 | 2 | 13.2266 | 0.7965 | 0.3586 | 0.1059 | 0.7313 | ns |
| Week_11 | MN | Route | 11.6677 | 2 | 11.8647 | 0.0016 | 0.8443 | 0.7023 | 0.9919 | ** |
| Week_12 | WN | Route | 0.3790 | 2 | 13.2137 | 0.6918 | 0.3645 | 0.0436 | 0.7253 | ns |
| Week_12 | MN | Route | 12.5813 | 2 | 12.6487 | 0.0010 | 0.8631 | 0.7246 | 1.0332 | *** |
| Week_13 | WN | Route | 0.3800 | 2 | 13.2733 | 0.6911 | 0.3427 | 0.0785 | 0.7311 | ns |
| Week_13 | MN | Route | 11.0540 | 2 | 11.9483 | 0.0019 | 0.8523 | 0.7450 | 0.9947 | ** |
| Week_14 | WN | Route | 0.1990 | 2 | 13.0343 | 0.8220 | 0.2911 | 0.0397 | 0.7604 | ns |
| Week_14 | MN | Route | 10.1230 | 2 | 12.6525 | 0.0024 | 0.8325 | 0.6854 | 1.0012 | ** |
| Week_15 | WN | Route | 1.2098 | 2 | 12.9990 | 0.3297 | 0.5001 | 0.1221 | 0.8663 | ns |
| Week_15 | MN | Route | 6.6894 | 2 | 10.4184 | 0.0135 | 0.8531 | 0.5907 | 1.0766 | * |
| Week_16 | WN | Route | 0.1823 | 2 | 13.0098 | 0.8354 | 0.3426 | 0.0731 | 0.7330 | ns |
| Week_16 | MN | Route | 7.9104 | 2 | 9.3879 | 0.0097 | 0.9453 | 0.7688 | 1.1793 | ** |

| | | | | | | | | | | |
|---------|----|-------|--------|---|---------|--------|--------|--------|--------|----|
| Week_17 | WN | Route | 0.1053 | 2 | 12.9985 | 0.9008 | 0.2752 | 0.0438 | 0.7046 | ns |
| Week_17 | MN | Route | 7.7361 | 2 | 9.5588 | 0.0100 | 0.9891 | 0.7925 | 1.2656 | * |
| Week_18 | WN | Route | 1.4892 | 2 | 11.5452 | 0.2659 | 0.4710 | 0.1032 | 0.8207 | ns |
| Week_18 | MN | Route | 4.0466 | 2 | 10.9429 | 0.0484 | 0.8530 | 0.4328 | 1.1840 | * |
| Week_19 | WN | Route | 0.1186 | 2 | 12.3347 | 0.8891 | 0.3585 | 0.0571 | 0.7342 | ns |
| Week_19 | MN | Route | 2.5349 | 2 | 10.7215 | 0.1255 | 0.7664 | 0.2584 | 1.2106 | ns |
| Week_20 | WN | Route | 0.3121 | 2 | 12.3417 | 0.7375 | 0.3788 | 0.0410 | 0.8568 | ns |
| Week_20 | MN | Route | 1.7845 | 2 | 11.1498 | 0.2126 | 0.7201 | 0.1120 | 1.2486 | ns |
| Week_21 | WN | Route | 0.9900 | 2 | 12.8920 | 0.3981 | 0.4395 | 0.0749 | 0.7919 | ns |
| Week_21 | MN | Route | 1.2693 | 2 | 12.1936 | 0.3157 | 0.5648 | 0.1110 | 0.9098 | ns |
| Week_22 | WN | Route | 1.1111 | 2 | 12.8371 | 0.3589 | 0.4654 | 0.1060 | 0.8061 | ns |
| Week_22 | MN | Route | 2.6553 | 2 | 11.3155 | 0.1134 | 0.7444 | 0.4043 | 1.2335 | ns |

Appendix Table 93
Robust one-way ANOVA - significance summary for Route effect

| Time_point | Factor | Effect | test | df1 | df2 | p.value | effsize | CI_lower | CI_upper | Sig. |
|------------|--------|--------|---------|-----|---------|---------|---------|----------|----------|------|
| Week_6 | MN | Route | 4.6012 | 2 | 12.5486 | 0.0317 | 0.7071 | 0.3539 | 0.9990 | * |
| Week_7 | MN | Route | 6.6197 | 2 | 11.8250 | 0.0118 | 0.7229 | 0.4533 | 0.9427 | * |
| Week_8 | MN | Route | 6.4573 | 2 | 12.2947 | 0.0121 | 0.7206 | 0.5076 | 0.9312 | * |
| Week_9 | MN | Route | 9.4920 | 2 | 12.4183 | 0.0032 | 0.7882 | 0.6314 | 0.9290 | ** |
| Week_10 | MN | Route | 7.8905 | 2 | 11.8254 | 0.0067 | 0.7947 | 0.5708 | 0.9749 | ** |
| Week_11 | MN | Route | 11.6677 | 2 | 11.8647 | 0.0016 | 0.8443 | 0.7023 | 0.9919 | ** |
| Week_12 | MN | Route | 12.5813 | 2 | 12.6487 | 0.0010 | 0.8631 | 0.7246 | 1.0332 | *** |
| Week_13 | MN | Route | 11.0540 | 2 | 11.9483 | 0.0019 | 0.8523 | 0.7450 | 0.9947 | ** |
| Week_14 | MN | Route | 10.1230 | 2 | 12.6525 | 0.0024 | 0.8325 | 0.6854 | 1.0012 | ** |
| Week_15 | MN | Route | 6.6894 | 2 | 10.4184 | 0.0135 | 0.8531 | 0.5907 | 1.0766 | * |
| Week_16 | MN | Route | 7.9104 | 2 | 9.3879 | 0.0097 | 0.9453 | 0.7688 | 1.1793 | ** |
| Week_17 | MN | Route | 7.7361 | 2 | 9.5588 | 0.0100 | 0.9891 | 0.7925 | 1.2656 | * |
| Week_18 | MN | Route | 4.0466 | 2 | 10.9429 | 0.0484 | 0.8530 | 0.4328 | 1.1840 | * |

For the pairwise comparison, we applied a Linear contrast expression. Since the “Diet” predictor only had two factor levels, the output showed the same result as the Robust one-way ANOVA above. For the “Route” predictor, the pairwise comparison presented a more detailed view at where statistically significant differences occurred (Appendix table 94). As for the Robust one-way ANOVA above, all statistical significant differences were observed between “Week_6” and “Week_18” and were restricted to the malnourished groups (Appendix table 95). The main differences between the malnourished groups resided primarily with the needle inoculated group from “Week_6” to “Week_14”, which had had more weight gain than either the malnourished control or sand fly infected groups. From “Week_15” onward, the average mouse weight for the malnourished control group approached that of the needle group and

statistical differences were now only observed compared to the malnourished sand fly infected group, that never seemed to gain weight post infection.

Appendix Table 94
Pairwise comparison by Linear Contrast Expression

| Time_point | Factor | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|----------------|---------|-------|---------|---------|----------|----------|---------|------|
| Split by Route | | | | | | | | |
| Week_0 | Control | WN | MN | -0.0113 | -0.0362 | 0.0136 | 0.3472 | ns |
| Week_0 | Needle | WN | MN | -0.0032 | -0.0301 | 0.0237 | 0.7921 | ns |
| Week_0 | SF | WN | MN | -0.0008 | -0.0216 | 0.0199 | 0.9334 | ns |
| Week_1 | Control | WN | MN | 1.1088 | 0.6678 | 1.5498 | 0.0002 | *** |
| Week_1 | Needle | WN | MN | 1.0070 | 0.6121 | 1.4019 | 0.0003 | *** |
| Week_1 | SF | WN | MN | 0.7394 | 0.3700 | 1.1089 | 0.0011 | ** |
| Week_2 | Control | WN | MN | 1.2031 | 0.8210 | 1.5852 | <0.0001 | **** |
| Week_2 | Needle | WN | MN | 1.0607 | 0.7290 | 1.3923 | <0.0001 | **** |
| Week_2 | SF | WN | MN | 0.8588 | 0.5315 | 1.1860 | 0.0001 | *** |
| Week_3 | Control | WN | MN | 1.4061 | 1.0452 | 1.7670 | <0.0001 | **** |
| Week_3 | Needle | WN | MN | 1.1827 | 0.8599 | 1.5055 | <0.0001 | **** |
| Week_3 | SF | WN | MN | 1.0654 | 0.7395 | 1.3913 | <0.0001 | **** |
| Week_4 | Control | WN | MN | 1.5285 | 1.1736 | 1.8833 | <0.0001 | **** |
| Week_4 | Needle | WN | MN | 1.0817 | 0.7741 | 1.3893 | <0.0001 | **** |
| Week_4 | SF | WN | MN | 1.0296 | 0.6955 | 1.3637 | <0.0001 | **** |
| Week_5 | Control | WN | MN | 1.4800 | 1.1601 | 1.7998 | <0.0001 | **** |
| Week_5 | Needle | WN | MN | 1.0373 | 0.7354 | 1.3392 | <0.0001 | **** |
| Week_5 | SF | WN | MN | 1.1033 | 0.7852 | 1.4215 | <0.0001 | **** |
| Week_6 | Control | WN | MN | 1.4642 | 1.1260 | 1.8023 | <0.0001 | **** |
| Week_6 | Needle | WN | MN | 1.0005 | 0.7049 | 1.2961 | <0.0001 | **** |
| Week_6 | SF | WN | MN | 1.3728 | 1.1210 | 1.6245 | <0.0001 | **** |
| Week_7 | Control | WN | MN | 1.5867 | 1.2853 | 1.8881 | <0.0001 | **** |
| Week_7 | Needle | WN | MN | 1.1054 | 0.8345 | 1.3762 | <0.0001 | **** |
| Week_7 | SF | WN | MN | 1.2398 | 0.9901 | 1.4895 | <0.0001 | **** |
| Week_8 | Control | WN | MN | 1.5293 | 1.2068 | 1.8519 | <0.0001 | **** |
| Week_8 | Needle | WN | MN | 0.9968 | 0.7274 | 1.2663 | <0.0001 | **** |
| Week_8 | SF | WN | MN | 1.3011 | 1.0055 | 1.5967 | <0.0001 | **** |
| Week_9 | Control | WN | MN | 1.5496 | 1.2641 | 1.8352 | <0.0001 | **** |
| Week_9 | Needle | WN | MN | 1.0374 | 0.7437 | 1.3311 | <0.0001 | **** |
| Week_9 | SF | WN | MN | 1.5182 | 1.2559 | 1.7805 | <0.0001 | **** |
| Week_10 | Control | WN | MN | 1.5132 | 1.2348 | 1.7917 | <0.0001 | **** |
| Week_10 | Needle | WN | MN | 0.9966 | 0.7071 | 1.2861 | <0.0001 | **** |
| Week_10 | SF | WN | MN | 1.4703 | 1.1779 | 1.7628 | <0.0001 | **** |

| | | | | | | | | |
|---------|---------|----|----|--------|--------|--------|---------|------|
| Week_11 | Control | WN | MN | 1.7096 | 1.4357 | 1.9835 | <0.0001 | **** |
| Week_11 | Needle | WN | MN | 1.0347 | 0.7412 | 1.3283 | <0.0001 | **** |
| Week_11 | SF | WN | MN | 1.6694 | 1.3810 | 1.9578 | <0.0001 | **** |
| Week_12 | Control | WN | MN | 1.6941 | 1.3671 | 2.0211 | <0.0001 | **** |
| Week_12 | Needle | WN | MN | 1.0008 | 0.7271 | 1.2745 | <0.0001 | **** |
| Week_12 | SF | WN | MN | 1.5285 | 1.2571 | 1.7998 | <0.0001 | **** |
| Week_13 | Control | WN | MN | 1.7818 | 1.4765 | 2.0870 | <0.0001 | **** |
| Week_13 | Needle | WN | MN | 1.0233 | 0.6838 | 1.3628 | <0.0001 | **** |
| Week_13 | SF | WN | MN | 1.6597 | 1.3356 | 1.9839 | <0.0001 | **** |
| Week_14 | Control | WN | MN | 1.5898 | 1.2118 | 1.9677 | <0.0001 | **** |
| Week_14 | Needle | WN | MN | 0.8714 | 0.5141 | 1.2287 | 0.0002 | *** |
| Week_14 | SF | WN | MN | 1.5966 | 1.2813 | 1.9118 | <0.0001 | **** |
| Week_15 | Control | WN | MN | 1.3339 | 1.0806 | 1.5871 | <0.0001 | **** |
| Week_15 | Needle | WN | MN | 0.9000 | 0.5145 | 1.2856 | 0.0003 | *** |
| Week_15 | SF | WN | MN | 1.6695 | 1.3417 | 1.9973 | <0.0001 | **** |
| Week_16 | Control | WN | MN | 1.3148 | 1.0152 | 1.6145 | <0.0001 | **** |
| Week_16 | Needle | WN | MN | 0.8993 | 0.5243 | 1.2743 | 0.0002 | *** |
| Week_16 | SF | WN | MN | 1.7211 | 1.3620 | 2.0801 | <0.0001 | **** |
| Week_17 | Control | WN | MN | 1.0999 | 0.8005 | 1.3993 | <0.0001 | **** |
| Week_17 | Needle | WN | MN | 0.9074 | 0.5154 | 1.2994 | 0.0003 | *** |
| Week_17 | SF | WN | MN | 1.6611 | 1.3056 | 2.0167 | <0.0001 | **** |
| Week_18 | Control | WN | MN | 1.3589 | 1.0769 | 1.6410 | <0.0001 | **** |
| Week_18 | Needle | WN | MN | 0.9492 | 0.5255 | 1.3729 | 0.0004 | *** |
| Week_18 | SF | WN | MN | 1.7918 | 1.4179 | 2.1656 | <0.0001 | **** |
| Week_19 | Control | WN | MN | 1.5472 | 1.2580 | 1.8364 | <0.0001 | **** |
| Week_19 | Needle | WN | MN | 1.2893 | 0.8758 | 1.7028 | <0.0001 | **** |
| Week_19 | SF | WN | MN | 1.7998 | 1.3412 | 2.2585 | <0.0001 | **** |
| Week_20 | Control | WN | MN | 1.7273 | 1.4320 | 2.0226 | <0.0001 | **** |
| Week_20 | Needle | WN | MN | 1.3372 | 0.8961 | 1.7784 | <0.0001 | **** |
| Week_20 | SF | WN | MN | 1.8728 | 1.4393 | 2.3063 | <0.0001 | **** |
| Week_21 | Control | WN | MN | 1.8837 | 1.5042 | 2.2633 | <0.0001 | **** |
| Week_21 | Needle | WN | MN | 1.3298 | 0.8195 | 1.8401 | 0.0001 | *** |
| Week_21 | SF | WN | MN | 1.8646 | 1.4923 | 2.2369 | <0.0001 | **** |
| Week_22 | Control | WN | MN | 1.7596 | 1.4338 | 2.0853 | <0.0001 | **** |
| Week_22 | Needle | WN | MN | 1.1723 | 0.6638 | 1.6807 | 0.0003 | *** |
| Week_22 | SF | WN | MN | 1.9461 | 1.5386 | 2.3537 | <0.0001 | **** |

Split by Diet

| | | | | | | | | |
|--------|----|---------|--------|---------|---------|--------|--------|----|
| Week_0 | WN | Control | Needle | 0.0125 | -0.0130 | 0.0381 | 0.2999 | ns |
| Week_0 | WN | Control | SF | -0.0028 | -0.0316 | 0.0259 | 0.7940 | ns |
| Week_0 | WN | Needle | SF | -0.0154 | -0.0387 | 0.0080 | 0.2847 | ns |

| | | | | | | | | |
|--------|----|---------|--------|---------|---------|---------|--------|----|
| Week_0 | MN | Control | Needle | 0.0206 | -0.0171 | 0.0584 | 0.4743 | ns |
| Week_0 | MN | Control | SF | 0.0077 | -0.0215 | 0.0368 | 0.4862 | ns |
| Week_0 | MN | Needle | SF | -0.0130 | -0.0487 | 0.0227 | 0.4862 | ns |
| Week_1 | WN | Control | Needle | 0.2286 | -0.4120 | 0.8692 | 0.3497 | ns |
| Week_1 | WN | Control | SF | 0.4935 | -0.1262 | 1.1133 | 0.1499 | ns |
| Week_1 | WN | Needle | SF | 0.2649 | -0.3182 | 0.8481 | 0.3497 | ns |
| Week_1 | MN | Control | Needle | 0.1268 | -0.2001 | 0.4536 | 0.4986 | ns |
| Week_1 | MN | Control | SF | 0.1242 | -0.2112 | 0.4595 | 0.4986 | ns |
| Week_1 | MN | Needle | SF | -0.0026 | -0.3095 | 0.3043 | 0.9820 | ns |
| Week_2 | WN | Control | Needle | 0.1875 | -0.3279 | 0.7030 | 0.3452 | ns |
| Week_2 | WN | Control | SF | 0.3592 | -0.1556 | 0.8741 | 0.2437 | ns |
| Week_2 | WN | Needle | SF | 0.1717 | -0.3048 | 0.6482 | 0.3452 | ns |
| Week_2 | MN | Control | Needle | 0.0451 | -0.3173 | 0.4074 | 0.9116 | ns |
| Week_2 | MN | Control | SF | 0.0148 | -0.3414 | 0.3711 | 0.9116 | ns |
| Week_2 | MN | Needle | SF | -0.0303 | -0.3601 | 0.2996 | 0.9116 | ns |
| Week_3 | WN | Control | Needle | 0.1814 | -0.3168 | 0.6796 | 0.4229 | ns |
| Week_3 | WN | Control | SF | 0.3088 | -0.1435 | 0.7612 | 0.2563 | ns |
| Week_3 | WN | Needle | SF | 0.1274 | -0.2962 | 0.5510 | 0.4229 | ns |
| Week_3 | MN | Control | Needle | -0.0420 | -0.3777 | 0.2936 | 0.9443 | ns |
| Week_3 | MN | Control | SF | -0.0319 | -0.4463 | 0.3824 | 0.9443 | ns |
| Week_3 | MN | Needle | SF | 0.0101 | -0.3856 | 0.4058 | 0.9443 | ns |
| Week_4 | WN | Control | Needle | 0.2594 | -0.2276 | 0.7464 | 0.2569 | ns |
| Week_4 | WN | Control | SF | 0.3197 | -0.1244 | 0.7638 | 0.2133 | ns |
| Week_4 | WN | Needle | SF | 0.0603 | -0.3414 | 0.4620 | 0.6858 | ns |
| Week_4 | MN | Control | Needle | -0.1874 | -0.5063 | 0.1314 | 0.4015 | ns |
| Week_4 | MN | Control | SF | -0.1792 | -0.6052 | 0.2468 | 0.4015 | ns |
| Week_4 | MN | Needle | SF | 0.0082 | -0.4045 | 0.4209 | 0.9562 | ns |
| Week_5 | WN | Control | Needle | 0.1664 | -0.2682 | 0.6009 | 0.4755 | ns |
| Week_5 | WN | Control | SF | 0.1940 | -0.2016 | 0.5895 | 0.4755 | ns |
| Week_5 | WN | Needle | SF | 0.0276 | -0.3392 | 0.3943 | 0.8391 | ns |
| Week_5 | MN | Control | Needle | -0.2763 | -0.6135 | 0.0609 | 0.1326 | ns |
| Week_5 | MN | Control | SF | -0.1827 | -0.5918 | 0.2264 | 0.3615 | ns |
| Week_5 | MN | Needle | SF | 0.0936 | -0.3203 | 0.5076 | 0.5426 | ns |
| Week_6 | WN | Control | Needle | 0.1055 | -0.3411 | 0.5520 | 0.7466 | ns |
| Week_6 | WN | Control | SF | 0.0515 | -0.3763 | 0.4794 | 0.7466 | ns |
| Week_6 | WN | Needle | SF | -0.0539 | -0.4140 | 0.3062 | 0.7466 | ns |
| Week_6 | MN | Control | Needle | -0.3582 | -0.7006 | -0.0157 | 0.0336 | * |
| Week_6 | MN | Control | SF | -0.0399 | -0.3543 | 0.2746 | 0.7359 | ns |
| Week_6 | MN | Needle | SF | 0.3183 | -0.0155 | 0.6521 | 0.0336 | * |
| Week_7 | WN | Control | Needle | 0.1199 | -0.3041 | 0.5439 | 0.4544 | ns |
| Week_7 | WN | Control | SF | 0.2430 | -0.1651 | 0.6510 | 0.3849 | ns |

| | | | | | | | | |
|---------|----|---------|--------|---------|---------|---------|--------|----|
| Week_7 | WN | Needle | SF | 0.1231 | -0.2224 | 0.4686 | 0.4544 | ns |
| Week_7 | MN | Control | Needle | -0.3614 | -0.6311 | -0.0918 | 0.0101 | * |
| Week_7 | MN | Control | SF | -0.1039 | -0.3720 | 0.1641 | 0.3033 | ns |
| Week_7 | MN | Needle | SF | 0.2575 | -0.0528 | 0.5679 | 0.0623 | + |
| Week_8 | WN | Control | Needle | 0.1235 | -0.2994 | 0.5463 | 0.6560 | ns |
| Week_8 | WN | Control | SF | 0.1682 | -0.2749 | 0.6113 | 0.6560 | ns |
| Week_8 | WN | Needle | SF | 0.0447 | -0.3199 | 0.4094 | 0.7439 | ns |
| Week_8 | MN | Control | Needle | -0.4090 | -0.7248 | -0.0932 | 0.0117 | * |
| Week_8 | MN | Control | SF | -0.0600 | -0.3788 | 0.2588 | 0.6141 | ns |
| Week_8 | MN | Needle | SF | 0.3490 | 0.0006 | 0.6974 | 0.0262 | * |
| Week_9 | WN | Control | Needle | 0.0432 | -0.3508 | 0.4372 | 0.8865 | ns |
| Week_9 | WN | Control | SF | 0.0626 | -0.3172 | 0.4424 | 0.8865 | ns |
| Week_9 | WN | Needle | SF | 0.0194 | -0.3437 | 0.3825 | 0.8865 | ns |
| Week_9 | MN | Control | Needle | -0.4690 | -0.7999 | -0.1381 | 0.0032 | ** |
| Week_9 | MN | Control | SF | 0.0312 | -0.2712 | 0.3335 | 0.7833 | ns |
| Week_9 | MN | Needle | SF | 0.5002 | 0.1608 | 0.8396 | 0.0032 | ** |
| Week_10 | WN | Control | Needle | 0.0840 | -0.3049 | 0.4729 | 0.8692 | ns |
| Week_10 | WN | Control | SF | 0.0835 | -0.3128 | 0.4798 | 0.8692 | ns |
| Week_10 | WN | Needle | SF | -0.0005 | -0.3659 | 0.3649 | 0.9970 | ns |
| Week_10 | MN | Control | Needle | -0.4326 | -0.7551 | -0.1101 | 0.0061 | ** |
| Week_10 | MN | Control | SF | 0.0406 | -0.2742 | 0.3554 | 0.7265 | ns |
| Week_10 | MN | Needle | SF | 0.4732 | 0.1053 | 0.8411 | 0.0061 | ** |
| Week_11 | WN | Control | Needle | 0.0946 | -0.2760 | 0.4651 | 0.7218 | ns |
| Week_11 | WN | Control | SF | 0.0489 | -0.3150 | 0.4128 | 0.7218 | ns |
| Week_11 | WN | Needle | SF | -0.0457 | -0.3762 | 0.2848 | 0.7218 | ns |
| Week_11 | MN | Control | Needle | -0.5803 | -0.9256 | -0.2350 | 0.0021 | ** |
| Week_11 | MN | Control | SF | 0.0087 | -0.3363 | 0.3537 | 0.9452 | ns |
| Week_11 | MN | Needle | SF | 0.5891 | 0.1917 | 0.9864 | 0.0023 | ** |
| Week_12 | WN | Control | Needle | 0.0957 | -0.3009 | 0.4923 | 0.7817 | ns |
| Week_12 | WN | Control | SF | 0.1294 | -0.2682 | 0.5269 | 0.7817 | ns |
| Week_12 | WN | Needle | SF | 0.0336 | -0.3029 | 0.3702 | 0.7901 | ns |
| Week_12 | MN | Control | Needle | -0.5976 | -0.9609 | -0.2343 | 0.0013 | ** |
| Week_12 | MN | Control | SF | -0.0363 | -0.3952 | 0.3225 | 0.7876 | ns |
| Week_12 | MN | Needle | SF | 0.5613 | 0.2112 | 0.9114 | 0.0013 | ** |
| Week_13 | WN | Control | Needle | 0.0787 | -0.3186 | 0.4761 | 0.6942 | ns |
| Week_13 | WN | Control | SF | 0.1318 | -0.2668 | 0.5304 | 0.6942 | ns |
| Week_13 | WN | Needle | SF | 0.0531 | -0.3059 | 0.4121 | 0.6942 | ns |
| Week_13 | MN | Control | Needle | -0.6798 | -1.0978 | -0.2617 | 0.0023 | ** |
| Week_13 | MN | Control | SF | 0.0098 | -0.3851 | 0.4047 | 0.9464 | ns |
| Week_13 | MN | Needle | SF | 0.6896 | 0.2244 | 1.1548 | 0.0023 | ** |
| Week_14 | WN | Control | Needle | 0.0949 | -0.3429 | 0.5328 | 0.8851 | ns |

| | | | | | | | | |
|---------|----|---------|--------|---------|---------|---------|--------|----|
| Week_14 | WN | Control | SF | 0.0816 | -0.3189 | 0.4821 | 0.8851 | ns |
| Week_14 | WN | Needle | SF | -0.0133 | -0.4111 | 0.3845 | 0.9282 | ns |
| Week_14 | MN | Control | Needle | -0.6234 | -1.1087 | -0.1382 | 0.0060 | ** |
| Week_14 | MN | Control | SF | 0.0884 | -0.3834 | 0.5602 | 0.6188 | ns |
| Week_14 | MN | Needle | SF | 0.7118 | 0.2609 | 1.1628 | 0.0029 | ** |
| Week_15 | WN | Control | Needle | 0.2235 | -0.1577 | 0.6047 | 0.4048 | ns |
| Week_15 | WN | Control | SF | 0.1138 | -0.2296 | 0.4572 | 0.4053 | ns |
| Week_15 | WN | Needle | SF | -0.1097 | -0.4595 | 0.2401 | 0.4053 | ns |
| Week_15 | MN | Control | Needle | -0.2103 | -0.6700 | 0.2494 | 0.2131 | ns |
| Week_15 | MN | Control | SF | 0.4494 | 0.0467 | 0.8521 | 0.0158 | * |
| Week_15 | MN | Needle | SF | 0.6597 | 0.1356 | 1.1839 | 0.0143 | * |
| Week_16 | WN | Control | Needle | 0.1005 | -0.3643 | 0.5653 | 0.8588 | ns |
| Week_16 | WN | Control | SF | 0.0285 | -0.3980 | 0.4550 | 0.8588 | ns |
| Week_16 | WN | Needle | SF | -0.0720 | -0.4872 | 0.3432 | 0.8588 | ns |
| Week_16 | MN | Control | Needle | -0.3150 | -0.7167 | 0.0866 | 0.0478 | * |
| Week_16 | MN | Control | SF | 0.4347 | 0.0119 | 0.8575 | 0.0243 | * |
| Week_16 | MN | Needle | SF | 0.7498 | 0.2463 | 1.2532 | 0.0045 | ** |
| Week_17 | WN | Control | Needle | 0.0257 | -0.4436 | 0.4950 | 0.8838 | ns |
| Week_17 | WN | Control | SF | -0.0446 | -0.4711 | 0.3820 | 0.8838 | ns |
| Week_17 | WN | Needle | SF | -0.0703 | -0.4944 | 0.3538 | 0.8838 | ns |
| Week_17 | MN | Control | Needle | -0.1668 | -0.5930 | 0.2594 | 0.2738 | ns |
| Week_17 | MN | Control | SF | 0.5167 | 0.1012 | 0.9322 | 0.0097 | ** |
| Week_17 | MN | Needle | SF | 0.6834 | 0.1699 | 1.1969 | 0.0097 | ** |
| Week_18 | WN | Control | Needle | 0.2123 | -0.2339 | 0.6586 | 0.3239 | ns |
| Week_18 | WN | Control | SF | -0.0312 | -0.3636 | 0.3013 | 0.7989 | ns |
| Week_18 | WN | Needle | SF | -0.2435 | -0.6451 | 0.1581 | 0.3239 | ns |
| Week_18 | MN | Control | Needle | -0.1974 | -0.6800 | 0.2852 | 0.2688 | ns |
| Week_18 | MN | Control | SF | 0.4017 | -0.0874 | 0.8907 | 0.0626 | + |
| Week_18 | MN | Needle | SF | 0.5990 | 0.0178 | 1.1803 | 0.0452 | * |
| Week_19 | WN | Control | Needle | 0.0355 | -0.4149 | 0.4858 | 0.8431 | ns |
| Week_19 | WN | Control | SF | 0.0648 | -0.2929 | 0.4226 | 0.8431 | ns |
| Week_19 | WN | Needle | SF | 0.0294 | -0.3806 | 0.4394 | 0.8431 | ns |
| Week_19 | MN | Control | Needle | -0.2224 | -0.6893 | 0.2444 | 0.2045 | ns |
| Week_19 | MN | Control | SF | 0.3175 | -0.2732 | 0.9083 | 0.2045 | ns |
| Week_19 | MN | Needle | SF | 0.5399 | -0.1059 | 1.1858 | 0.1208 | ns |
| Week_20 | WN | Control | Needle | 0.1231 | -0.2939 | 0.5401 | 0.7165 | ns |
| Week_20 | WN | Control | SF | 0.0453 | -0.2885 | 0.3791 | 0.7165 | ns |
| Week_20 | WN | Needle | SF | -0.0778 | -0.4556 | 0.3000 | 0.7165 | ns |
| Week_20 | MN | Control | Needle | -0.2670 | -0.8043 | 0.2703 | 0.2826 | ns |
| Week_20 | MN | Control | SF | 0.1908 | -0.3773 | 0.7588 | 0.3594 | ns |
| Week_20 | MN | Needle | SF | 0.4578 | -0.2003 | 1.1158 | 0.2396 | ns |

| | | | | | | | | |
|---------|----|---------|--------|---------|---------|--------|--------|----|
| Week_21 | WN | Control | Needle | 0.2520 | -0.2269 | 0.7309 | 0.5255 | ns |
| Week_21 | WN | Control | SF | 0.0955 | -0.3169 | 0.5080 | 0.5417 | ns |
| Week_21 | WN | Needle | SF | -0.1565 | -0.6057 | 0.2927 | 0.5331 | ns |
| Week_21 | MN | Control | Needle | -0.3019 | -0.9467 | 0.3428 | 0.3291 | ns |
| Week_21 | MN | Control | SF | 0.0764 | -0.4447 | 0.5976 | 0.6962 | ns |
| Week_21 | MN | Needle | SF | 0.3784 | -0.2771 | 1.0338 | 0.3291 | ns |
| Week_22 | WN | Control | Needle | 0.2645 | -0.2216 | 0.7507 | 0.3912 | ns |
| Week_22 | WN | Control | SF | 0.0605 | -0.3400 | 0.4611 | 0.6904 | ns |
| Week_22 | WN | Needle | SF | -0.2040 | -0.6813 | 0.2733 | 0.3912 | ns |
| Week_22 | MN | Control | Needle | -0.3228 | -0.9302 | 0.2847 | 0.2120 | ns |
| Week_22 | MN | Control | SF | 0.2471 | -0.2738 | 0.7679 | 0.2120 | ns |
| Week_22 | MN | Needle | SF | 0.5698 | -0.0941 | 1.2338 | 0.1082 | ns |

Appendix Table 95
Pairwise comparison by Linear Contrast Expression - sig. summary

| Predictor | Time_point | Factor | Group | Group.1 | psihat | ci.lower | ci.upper | p.value | Sig. |
|-----------|------------|--------|---------|---------|---------|----------|----------|---------|------|
| Route | Week_6 | MN | Control | Needle | -0.3582 | -0.7006 | -0.0157 | 0.0336 | * |
| Route | Week_6 | MN | Needle | SF | 0.3183 | -0.0155 | 0.6521 | 0.0336 | * |
| Route | Week_7 | MN | Control | Needle | -0.3614 | -0.6311 | -0.0918 | 0.0101 | * |
| Route | Week_8 | MN | Control | Needle | -0.4090 | -0.7248 | -0.0932 | 0.0117 | * |
| Route | Week_8 | MN | Needle | SF | 0.3490 | 0.0006 | 0.6974 | 0.0262 | * |
| Route | Week_9 | MN | Control | Needle | -0.4690 | -0.7999 | -0.1381 | 0.0032 | ** |
| Route | Week_9 | MN | Needle | SF | 0.5002 | 0.1608 | 0.8396 | 0.0032 | ** |
| Route | Week_10 | MN | Control | Needle | -0.4326 | -0.7551 | -0.1101 | 0.0061 | ** |
| Route | Week_10 | MN | Needle | SF | 0.4732 | 0.1053 | 0.8411 | 0.0061 | ** |
| Route | Week_11 | MN | Control | Needle | -0.5803 | -0.9256 | -0.2350 | 0.0021 | ** |
| Route | Week_11 | MN | Needle | SF | 0.5891 | 0.1917 | 0.9864 | 0.0023 | ** |
| Route | Week_12 | MN | Control | Needle | -0.5976 | -0.9609 | -0.2343 | 0.0013 | ** |
| Route | Week_12 | MN | Needle | SF | 0.5613 | 0.2112 | 0.9114 | 0.0013 | ** |
| Route | Week_13 | MN | Control | Needle | -0.6798 | -1.0978 | -0.2617 | 0.0023 | ** |
| Route | Week_13 | MN | Needle | SF | 0.6896 | 0.2244 | 1.1548 | 0.0023 | ** |
| Route | Week_14 | MN | Control | Needle | -0.6234 | -1.1087 | -0.1382 | 0.0060 | ** |
| Route | Week_14 | MN | Needle | SF | 0.7118 | 0.2609 | 1.1628 | 0.0029 | ** |
| Route | Week_15 | MN | Control | SF | 0.4494 | 0.0467 | 0.8521 | 0.0158 | * |
| Route | Week_15 | MN | Needle | SF | 0.6597 | 0.1356 | 1.1839 | 0.0143 | * |
| Route | Week_16 | MN | Control | Needle | -0.3150 | -0.7167 | 0.0866 | 0.0478 | * |
| Route | Week_16 | MN | Control | SF | 0.4347 | 0.0119 | 0.8575 | 0.0243 | * |
| Route | Week_16 | MN | Needle | SF | 0.7498 | 0.2463 | 1.2532 | 0.0045 | ** |
| Route | Week_17 | MN | Control | SF | 0.5167 | 0.1012 | 0.9322 | 0.0097 | ** |

| | | | | | | | | | |
|-------|---------|----|--------|----|--------|--------|--------|--------|----|
| Route | Week_17 | MN | Needle | SF | 0.6834 | 0.1699 | 1.1969 | 0.0097 | ** |
| Route | Week_18 | MN | Needle | SF | 0.5990 | 0.0178 | 1.1803 | 0.0452 | * |

Conclusion

In conclusion, “Diet” was identified of being the most potent predictor for mouse weight gain over time. Interestingly, between the well-nourished groups, we never observed statistically significant differences in weight gains over time, suggesting that in that state, and conversely to the malnourished mouse groups, mouse weight was not affected by infection status or “Route”. These data support the hypothesis that the nutritional state of an individual can directly impact their weight before and during *Leishmania donovani* infection.

Panel b

Data analysis

We analyzed a total of $N=98$ well-nourished (WN) and malnourished (MN) BALB/c mice for the occurrence frequency of a $\geq 20\%$ weight loss post-intradermal *Leishmania donovani* infection either by “needle” injection or sand fly bite (SF) (N : MN_Needle=20, MN_SF=34, WN_Needle=10, WN_SF=34) by contingency table analysis and logistic regression.

Contingency table

Due to the small sample sizes of some groups, there were several expected counts <5 , why we opted for the Fisher’s Exact test, which had the added benefit of producing exact p-value calculation. The analysis rendered a p-value of 0.00519, suggesting a statistically significant difference between groups. This was confirmed by the pairwise Fisher’s Exact test corrected by the Benjamin-Hochberg method, although the only statistically significant difference was observed for well-nourished and malnourished sand fly infected mice (Appendix table 96).

Appendix Table 96
Pairwise Fisher’s Exact test

| group1 | group2 | n | estimate | p | conf.low | conf.high | alternative | p.adj | p.adj.signif |
|-----------|-----------|----|----------|--------|----------|-----------|-------------|--------|--------------|
| MN_Needle | MN_SF | 54 | 2.7223 | 0.2910 | 0.4647 | 29.2690 | two.sided | 0.4360 | ns |
| MN_Needle | WN_Needle | 30 | 0.0000 | 0.5400 | 0.0000 | 10.8041 | two.sided | 0.6480 | ns |
| MN_Needle | WN_SF | 54 | 0.0000 | 0.1330 | 0.0000 | 3.0783 | two.sided | 0.3340 | ns |
| MN_SF | WN_Needle | 44 | 0.0000 | 0.1670 | 0.0000 | 1.8885 | two.sided | 0.3340 | ns |
| MN_SF | WN_SF | 68 | 0.0000 | 0.0049 | 0.0000 | 0.5050 | two.sided | 0.0295 | * |
| WN_Needle | WN_SF | 44 | 0.0000 | 1.0000 | 0.0000 | Inf | two.sided | 1.0000 | ns |

The observed odds ratios suggested that well-nourished BALB/c mice had a 0-fold likelihood of developing

a $\geq 20\%$ weight loss due to *L. donovani* infection, regardless of the infection route (Appendix table 97). Conversely, malnourished animals did develop the weight loss post infection. Although the malnourished mice infected by sand fly bite, did show a greater occurrence rate of $\geq 20\%$ weight loss compared to needle inoculated mice, that difference did not achieve statistical significance. The retrospective sample size and power calculations showed that our sample was sufficiently large enough at the total N to have sufficient power, the fact that the sample size per group were not equal (WN_needle only contained 10 mice) affected the actual test power (Appendix table 98). Either way, the contingency analysis suggested that “Diet”, rather than infection “Route” was key in the occurrence of critical weight loss post infection.

Appendix Table 97

Odds Ratios

| Groups | estimate | lower | upper | p.value |
|-----------|----------|---------|---------|---------|
| MN_Needle | 1.0000 | NA | NA | NA |
| MN_SF | 2.7223 | 0.4647 | 29.2690 | 0.2912 |
| WN_Needle | <0.0001 | <0.0001 | 10.8041 | 0.5402 |
| WN_SF | <0.0001 | <0.0001 | 3.0783 | 0.1328 |

Appendix Table 98

Retrospective Power Calculation

| Parameters | Calculation for | |
|--------------------------|-----------------|-------------------|
| | Sample size | Statistical power |
| Statistical power | 0.8 | 0.826 |
| Total n | 93 | 98 |
| Degrees of freedom | 3 | 3 |
| Non-centrality parameter | 10.903 | 11.59 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.174 |

Logistic regression

Due to the lack of events in the well-nourished group, logistic regression was not possible here as it rendered nonsensical data due to its dependence on the maximum likelihood estimation, which rendered an infinite estimate under these circumstance.

Panel c

Here, we analyzed the occurrence of ocular pathology following *Leishmania donovani* infection in well-nourished (WN) and malnourished (MN) BALB/c mice. A total of $N=98$ BALB/c mice (WN_Needle=10, MN_Needle=20, MN_SF=34, WN_SF=34) were examined on a weekly bases post infection by either “needle” or sand fly (SF) route and occurrence of pathology was recorded as time-to-event data.

Survival analysis

The data was analyzed by the Mantel-Haenszel's log-rank test by use of the `survdif()` function from the survival package in R. The test output is shown in appendix table 99, which was statistically significant ($F(3)=95.73$, <0.0001).

Appendix Table 99
Log-rank test

| Groups | N | Observed | Expected | Chi-Square | log-rank |
|-----------|----|----------|----------|-----------------------|-----------------------|
| | | | | (O-E) ² /E | (O-E) ² /V |
| WN_Needle | 10 | 0 | 3.8551 | 3.8551 | 4.5619 |
| WN_SF | 34 | 0 | 13.1074 | 13.1074 | 23.3213 |
| MN_Needle | 20 | 2 | 7.5019 | 4.0351 | 5.4924 |
| MN_SF | 34 | 30 | 7.5356 | 66.9693 | 95.3186 |

The pairwise Mantel-Haenszel' log-rank test, which was adjusted by the Benjamin-Hochberg correction, showed that the malnourished, sand fly inoculated group was statistically significant from all other groups, while there was no statistically significant difference between the remaining three groups (Appendix table 100).

Appendix Table 100
Pairwise Log-rank test

| Groups | WN_Needle | WN_SF | MN_Needle |
|-----------|-----------|---------|-----------|
| WN_SF | 1.0000 | NA | NA |
| MN_Needle | 0.3733 | 0.0927 | NA |
| MN_SF | <0.0001 | <0.0001 | <0.0001 |

In fact, only malnourished animals developed ocular pathology post infection and the grand majority of these were inoculated by sand fly (Appendix table 101), suggesting that sand fly bites significantly increased the occurrence of ocular pathology in malnourished hosts.

Appendix Table 101
Ocular pathology occurrence rate

| | Well-nourished | | Malnourished | |
|------------------|----------------|----------|--------------|----------|
| | Needle | Sand fly | Needle | Sand fly |
| Group N | 10 | 34 | 20 | 34 |
| Ocular Pathology | 0 | 0 | 2 | 30 |

| | | | | |
|----------------|-------|-------|--------|--------|
| Occurance Rate | 0.00% | 0.00% | 10.00% | 88.24% |
|----------------|-------|-------|--------|--------|

Cox proportional hazards regression model

We also explored the data by Cox proportional hazards regression. Due to the lack of events in the well-nourished group, we had to resort to Firth's penalized maximum likelihood bias reduction method for Cox regression. The output showed that both predictors, "Diet" and "Route", were statistically significant (Appendix table 102).

Appendix Table 102

Firth's penalized maximum likelihood bias reduction method for Cox regression

| Groups | coef | se(coef) | exp(coef) | lower 0.95 | upper 0.95 | Chisq | p | sig. |
|---------|------|----------|-----------|------------|------------|-------|---------|------|
| DietMN | 4.89 | 1.47 | 133.59 | 18.59 | 16969.83 | 62.67 | <0.0001 | **** |
| RouteSF | 2.70 | 0.68 | 14.83 | 4.83 | 73.44 | 31.60 | <0.0001 | **** |

The odds ratios suggested that "Diet" was a much more potent predictor for ocular pathology, than "Route", although both were statistically significant. Malnourished mice were about 133.6-times more likely to develop ocular pathology than well-nourished mice compared to sand fly inoculated mice being 14.8-times more likely to developed ocular pathology compared to the once inoculated by needle (Appendix table 103).

Appendix Table 103

Odds ratios

| Groups | AHR | 2.5 % | 97.5 % | p-value | sig. |
|---------|--------|-------|---------|---------|------|
| DietMN | 133.59 | 7.52 | 2373.94 | <0.0001 | **** |
| RouteSF | 14.83 | 3.89 | 56.51 | <0.0001 | **** |

The pairwise comparison clearly confirmed the observation of the log-rank test that the malnourished, sand fly inoculated group was statistically significantly different from the other three groups (Appendix table 104 and 105). Here, the analysis also separated well-nourished needle inoculated mice from the other three groups, which may be due to the small samples.

Appendix Table 104

Pariwise comparison by estimated marginal means

| Groups | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------------|----------|------|-----|---------|---------|------|
| WN Needle - MN Needle | -4.89 | 1.47 | Inf | -3.33 | 0.0051 | ** |
| WN Needle - WN SF | -2.70 | 0.68 | Inf | -3.95 | 0.0005 | *** |
| WN Needle - MN SF | -7.59 | 1.64 | Inf | -4.63 | <0.0001 | **** |

| | | | | | | |
|-------------------|-------|------|-----|-------|--------|-----|
| MN Needle - WN SF | 2.20 | 1.60 | Inf | 1.38 | 0.6697 | ns |
| MN Needle - MN SF | -2.70 | 0.68 | Inf | -3.95 | 0.0005 | *** |
| WN SF - MN SF | -4.89 | 1.47 | Inf | -3.33 | 0.0051 | ** |

Appendix Table 105
Pariwise comparison by estimated marginal means (letters)

| Diet | Route | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|------|--------|--------|------|-----|------------|------------|--------|
| WN | Needle | 0.00 | 0.00 | Inf | 0.00 | 0.00 | a |
| WN | SF | 2.70 | 0.68 | Inf | 1.00 | 4.40 | b |
| MN | Needle | 4.89 | 1.47 | Inf | 1.24 | 8.55 | b |
| MN | SF | 7.59 | 1.64 | Inf | 3.50 | 11.68 | c |

Panel d

Here, we present the parasite counts from several isolated tissues (brain, ears, eyes, liver, paw and spleen) according to qPCR as a measure of parasite dissemination to these tissues. To analyze the data, we had to re-scale it, due to the frequent occurrence of zero-values due to instances of no detection, by dividing all values by the smallest non-zero value in the dataset. This resulted in an approximate Poisson / negative binomial distribution, which allowed the convenient analysis of the re-scaled and rounded counts by the appropriate models for these distributions.

We analyzed varying total N of the different tissue samples (Appendix table 106). Total N and group N were dependent on available animals. While the spleen was always collected, other tissue types were only considered later in the study, as to why their total N is much lower. In general, a zero-inflated negative binomial model was the best fit our data, with the exception of eye and liver sample, where a zero-inflated Poisson and standard Poisson regression model fitted best, respectively. Model fit was assessed by overdispersion test and pseudo r^2 .

Appendix Table 106
Summary information

| Tissue | Group N | | | | Selected model | | Overdispersion test | | |
|--------|---------|-----------|-------|-------|-------------------|--------------------|---------------------|---------|-----------------------|
| | Total N | MN_Needle | WN_SF | MN_SF | Type ¹ | Model ² | Ratio | p_value | Pseudo R ² |
| Brain | 26 | 7 | 8 | 11 | zero-inf. | NB | 1.1272 | 0.6160 | 0.0578 |
| Ear | 38 | 11 | 12 | 15 | zero-inf. | NB | 2.1435 | 0.2400 | 0.3773 |
| Eye | 26 | 7 | 8 | 11 | zero-inf. | Poisson | 1.3351 | 0.4160 | 0.2482 |
| Liver | 26 | 7 | 8 | 11 | standard | Poisson | 1.0000 | 0.4608 | 0.3126 |
| Paw | 26 | 7 | 8 | 11 | zero-inf. | NB | 0.6729 | 0.5440 | 0.1567 |
| Spleen | 85 | 17 | 32 | 36 | zero-inf. | NB | 3.1538 | 0.0800 | 0.2736 |

¹zero-inf. = zero-inflated

²NB = negative binomial

The summary of the best fitting models per tissue, according to dispersion test, rather than AIC in this case, is shown in appendix table 107. In general, malnourished, needle inoculated mice served as the reference sample in the regression analysis. Statistical significance was only observed for ear and spleen.

Appendix Table 107

Model summary outputs

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|---------------|----------|------------|---------|----------|------|
| Brain | | | | | |
| (Intercept) | 3.5699 | 0.7886 | 4.5270 | <0.0001 | **** |
| DietMN_SF | -0.5966 | 0.9339 | -0.6388 | 0.5230 | ns |
| DietWN_SF | -1.3865 | 1.0766 | -1.2879 | 0.1978 | ns |
| Ear | | | | | |
| (Intercept) | 1.6452 | 0.5816 | 2.8289 | 0.0047 | ** |
| DietMN_SF | 1.7824 | 0.7586 | 2.3494 | 0.0188 | * |
| DietWN_SF | 4.0175 | 0.7953 | 5.0518 | <0.0001 | **** |
| Eye | | | | | |
| (Intercept) | -20.8473 | 18851.5363 | -0.0011 | 0.9991 | ns |
| DietMN_SF | 21.1547 | 18851.5363 | 0.0011 | 0.9991 | ns |
| DietWN_SF | 21.8020 | 18851.5363 | 0.0012 | 0.9991 | ns |
| Liver | | | | | |
| (Intercept) | -19.3026 | 3562.9263 | -0.0054 | 0.9957 | ns |
| DietMN_SF | 18.6964 | 3562.9263 | 0.0052 | 0.9958 | ns |
| DietWN_SF | 17.2231 | 3562.9264 | 0.0048 | 0.9961 | ns |
| Paw | | | | | |
| (Intercept) | -0.1928 | 2.9165 | -0.0661 | 0.9473 | ns |
| DietMN_SF | 3.3461 | 1.8853 | 1.7748 | 0.0759 | + |
| DietWN_SF | -1.5465 | 2.2330 | -0.6926 | 0.4886 | ns |
| Spleen | | | | | |
| (Intercept) | 0.8303 | 0.6791 | 1.2227 | 0.2214 | ns |
| DietMN_SF | 4.2820 | 0.8167 | 5.2431 | <0.0001 | **** |
| DietWN_SF | 0.4489 | 0.8374 | 0.5360 | 0.5919 | ns |

We applied a pairwise comparison based on the estimated marginal means for each tissue, respectively

(Appendix table 107 and 108). The analysis showed no statistically significant difference between groups for the tissues brain, eye and liver. For the paw, we observed a statistically significant difference between well-nourished and malnourished sand fly inoculated mice, but the result may not be reliable due to the small sample size. For the ear samples we observed statistically significant difference between all pairs with the well-nourished, sand fly inoculated mice clustering most strongly away from either malnourished group. In case of the spleen, it was the malnourished, sand fly inoculated group that was statistically significantly different from the other two groups, suggesting significantly higher and more frequent occurrence of parasites in spleen in this group.

Appendix Table 108
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-------------------|----------|------------|-----|---------|---------|------|
| Brain | | | | | | |
| MN_Needle - MN_SF | 0.5966 | 0.9339 | Inf | 0.6388 | 0.523 | ns |
| MN_Needle - WN_SF | 1.3865 | 1.0766 | Inf | 1.2879 | 0.523 | ns |
| MN_SF - WN_SF | 0.7899 | 0.9768 | Inf | 0.8087 | 0.523 | ns |
| Ear | | | | | | |
| MN_Needle - MN_SF | -1.7824 | 0.7586 | Inf | -2.3494 | 0.0188 | * |
| MN_Needle - WN_SF | -4.0175 | 0.7953 | Inf | -5.0518 | <0.0001 | **** |
| MN_SF - WN_SF | -2.2352 | 0.7291 | Inf | -3.0657 | 0.0033 | ** |
| Eye | | | | | | |
| MN_Needle - MN_SF | -21.1547 | 18851.5363 | Inf | -0.0011 | 0.9991 | ns |
| MN_Needle - WN_SF | -21.8020 | 18851.5363 | Inf | -0.0012 | 0.9991 | ns |
| MN_SF - WN_SF | -0.6473 | 0.6414 | Inf | -1.0093 | 0.9385 | ns |
| Liver | | | | | | |
| MN_Needle - MN_SF | -18.6964 | 3562.9263 | Inf | -0.0052 | 0.9961 | ns |
| MN_Needle - WN_SF | -17.2231 | 3562.9264 | Inf | -0.0048 | 0.9961 | ns |
| MN_SF - WN_SF | 1.4733 | 1.0801 | Inf | 1.3640 | 0.5177 | ns |
| Paw | | | | | | |
| MN_Needle - MN_SF | -3.3461 | 1.8853 | Inf | -1.7748 | 0.1139 | ns |
| MN_Needle - WN_SF | 1.5465 | 2.2330 | Inf | 0.6926 | 0.4886 | ns |
| MN_SF - WN_SF | 4.8926 | 2.0285 | Inf | 2.4119 | 0.0476 | * |
| Spleen | | | | | | |
| MN_Needle - MN_SF | -4.2820 | 0.8167 | Inf | -5.2431 | <0.0001 | **** |
| MN_Needle - WN_SF | -0.4489 | 0.8374 | Inf | -0.5360 | 0.5919 | ns |

| | | | | | | |
|---------------|--------|--------|-----|--------|---------|------|
| MN_SF - WN_SF | 3.8331 | 0.6678 | Inf | 5.7403 | <0.0001 | **** |
|---------------|--------|--------|-----|--------|---------|------|

Appendix Table 109
Pairwise comparison letter code

| Predictor levels | emmean | SE | df | asyp.LCL | asyp.UCL | .group |
|------------------|----------|------------|-----|-------------|------------|--------|
| Brain | | | | | | |
| WN_SF | 2.1834 | 0.8822 | Inf | 0.0713 | 4.2954 | a |
| MN_SF | 2.9733 | 0.6029 | Inf | 1.5300 | 4.4166 | a |
| MN_Needle | 3.5699 | 0.7886 | Inf | 1.6820 | 5.4577 | a |
| Ear | | | | | | |
| MN_Needle | 1.6452 | 0.5816 | Inf | 0.2529 | 3.0374 | a |
| MN_SF | 3.4275 | 0.4872 | Inf | 2.2613 | 4.5938 | b |
| WN_SF | 5.6627 | 0.5424 | Inf | 4.3641 | 6.9612 | c |
| Eye | | | | | | |
| MN_Needle | -20.8473 | 18851.5363 | Inf | -45151.0445 | 45109.3499 | a |
| MN_SF | 0.3074 | 0.5689 | Inf | -1.0544 | 1.6692 | a |
| WN_SF | 0.9547 | 0.3263 | Inf | 0.1735 | 1.7360 | a |
| Liver | | | | | | |
| MN_Needle | -19.3026 | 3562.9263 | Inf | -8548.8761 | 8510.2709 | a |
| WN_SF | -2.0794 | 1.0000 | Inf | -4.4734 | 0.3145 | a |
| MN_SF | -0.6061 | 0.4082 | Inf | -1.5835 | 0.3712 | a |
| Paw | | | | | | |
| WN_SF | -1.7393 | 2.8074 | Inf | -8.4601 | 4.9816 | a |
| MN_Needle | -0.1928 | 2.9165 | Inf | -7.1748 | 6.7893 | ab |
| MN_SF | 3.1533 | 2.8624 | Inf | -3.6992 | 10.0059 | b |
| Spleen | | | | | | |
| MN_Needle | 0.8303 | 0.6791 | Inf | -0.7954 | 2.4561 | a |
| WN_SF | 1.2792 | 0.4900 | Inf | 0.1062 | 2.4522 | a |
| MN_SF | 5.1123 | 0.4537 | Inf | 4.0262 | 6.1984 | b |

Panel e

Data analysis

We analysed the frequency of *Leishmania donovani* dissemination to several different tissue sites (brain, ears, eyes, liver, paw and spleen) in a varying total *N* of well-nourished (WN) and malnourished (MN)

BALB/c mice infected intradermally either by “needle” injection or sand fly bite (SF) (Appendix table 110). We used contingency table analysis and logistic regression here. These are the same animals as presented in figure 3d. These analyses permitted looking at our data from another angle to fully comprehend the impact of infection route and state of nourishment on parasite dissemination.

Appendix Table 110

Sample size

| Tissue | Total N | MN_Needle | MN_SF | WN_SF |
|--------|---------|-----------|-------|-------|
| Brain | 26 | 7 | 11 | 8 |
| Ear | 38 | 11 | 15 | 12 |
| Eye | 26 | 7 | 11 | 8 |
| Liver | 26 | 7 | 11 | 8 |
| Paw | 26 | 7 | 11 | 8 |
| Spleen | 83 | 17 | 34 | 32 |

Contingency table

Due to the small sample sizes in most datasets, there were several expected counts <5 , why we opted for the Fisher’s Exact test, which had the added benefit of exact p-value calculation, for all tissues except the spleen, where the sample size was much larger and a Chi-square test was applied. Only the spleen showed a statistical significant difference (Appendix table 111).

Appendix Table 111

Contingency table analyses

| Tissue | n | p | p.signif | Test |
|--------|----|--------|----------|---------------------|
| Brain | 26 | 0.5410 | ns | Fisher’s Exact test |
| Ear | 38 | 0.8870 | ns | Fisher’s Exact test |
| Eye | 26 | 0.1120 | ns | Fisher’s Exact test |
| Liver | 26 | 0.1750 | ns | Fisher’s Exact test |
| Paw | 26 | 0.8360 | ns | Fisher’s Exact test |
| Spleen | 83 | 0.0446 | * | Chi-square test |

Interestingly, the pairwise comparison did not show any statistical significant difference, even before adjusting p-values for multiple comparisons (Appendix table 112), suggesting that there is no statistically significant difference between groups within any tissue in terms of dissemination success.

Appendix Table 112

Pairwise comparison

| group | n | estimate | p | p.adj | p.adj.signif |
|-----------------------------|----|----------|--------|-------|--------------|
| Brain - Fisher's Exact test | | | | | |
| MN_Needle - MN_SF | 18 | 1.2926 | 1.0000 | 1.000 | ns |
| MN_Needle - WN_SF | 15 | 0.4753 | 0.6190 | 0.928 | ns |
| MN_SF - WN_SF | 19 | 0.3638 | 0.3700 | 0.928 | ns |
| Liver - Fisher's Exact test | | | | | |
| MN_Needle - MN_SF | 18 | Inf | 0.1190 | 0.357 | ns |
| MN_Needle - WN_SF | 15 | Inf | 1.0000 | 1.000 | ns |
| MN_SF - WN_SF | 19 | 0.2678 | 0.3380 | 0.507 | ns |
| Paw - Fisher's Exact test | | | | | |
| MN_Needle - MN_SF | 18 | 2.1565 | 1.0000 | 1.000 | ns |
| MN_Needle - WN_SF | 15 | 0.8660 | 1.0000 | 1.000 | ns |
| MN_SF - WN_SF | 19 | 0.3997 | 0.6030 | 1.000 | ns |
| Spleen - Chi-square test | | | | | |
| MN_Needle - MN_SF | 51 | 3.5406 | 0.0599 | 0.127 | ns |
| MN_Needle - WN_SF | 49 | 0.0630 | 0.8020 | 0.802 | ns |
| MN_SF - WN_SF | 66 | 2.9724 | 0.0847 | 0.127 | ns |
| Ear - Fisher's Exact test | | | | | |
| MN_Needle - MN_SF | 26 | 1.4763 | 1.0000 | 1.000 | ns |
| MN_Needle - WN_SF | 23 | 1.8241 | 0.6400 | 1.000 | ns |
| MN_SF - WN_SF | 27 | 1.2398 | 1.0000 | 1.000 | ns |
| Eye - Fisher's Exact test | | | | | |
| MN_Needle - MN_SF | 18 | Inf | 0.2450 | 0.368 | ns |
| MN_Needle - WN_SF | 15 | Inf | 0.0769 | 0.231 | ns |
| MN_SF - WN_SF | 19 | 2.5255 | 0.3770 | 0.377 | ns |

Conversely, when looking at the odds ratios, malnourished, sand fly inoculated mice were significantly more likely to experience parasite dissemination to the spleen than malnourished, needle inoculated mice, which served as the reference group in this analysis (Appendix table 113).

Appendix Table 113
Odds Ratios

| Groups | estimate | lower | upper | p.value | sig. |
|-----------|----------|-------|-------|---------|------|
| Brain | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |

| | | | | | |
|-----------|--------|--------|----------|--------|----|
| MN_SF | 1.2926 | 0.1220 | 13.1405 | 1.0000 | ns |
| WN_SF | 0.4753 | 0.0362 | 5.2820 | 0.6193 | ns |
| Ear | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |
| MN_SF | 1.4763 | 0.1562 | 14.0607 | 1.0000 | ns |
| WN_SF | 1.8241 | 0.1648 | 26.9593 | 0.6404 | ns |
| Eye | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |
| MN_SF | Inf | 0.2687 | Inf | 0.2451 | ns |
| WN_SF | Inf | 0.6991 | Inf | 0.0769 | + |
| Liver | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |
| MN_SF | Inf | 0.4574 | Inf | 0.1193 | ns |
| WN_SF | Inf | 0.0225 | Inf | 1.0000 | ns |
| Paw | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |
| MN_SF | 2.1565 | 0.1311 | 137.3685 | 1.0000 | ns |
| WN_SF | 0.8660 | 0.0096 | 78.3189 | 1.0000 | ns |
| Spleen | | | | | |
| MN_Needle | 1.0000 | NA | NA | NA | NA |
| MN_SF | 3.7196 | 1.0912 | 14.4367 | 0.0399 | * |
| WN_SF | 1.4147 | 0.4021 | 5.5178 | 0.7543 | ns |

Applying a retrospective statistical power calculation showed that the sample size for most tissues was too small to detect a statistical difference and thus, our statistical power was well below the standard 80% for all tissues (Appendix table 114). This observation suggested that the lack of observing statistical significance may be due to type II errors. However, larger sample sizes were prohibitive due to cost and loss of life.

Appendix Table 114
Retrospective Power Calculation

| Parameters | Calculation for | |
|-------------------|-----------------|-------------------|
| | Sample size | Statistical power |
| Brain | | |
| Statistical power | 0.8 | 0.161 |

| | | |
|--------------------------|------------|--------------|
| Total n | 191 | 26 |
| Degrees of freedom | 2 | 2 |
| Non-centrality parameter | 9.635 | 1.315 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.839 |
| Ear | | |
| Statistical power | 0.8 | 0.082 |
| Total n | 905 | 38 |
| Degrees of freedom | 2 | 2 |
| Non-centrality parameter | 9.635 | 0.405 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.918 |
| Eye | | |
| Statistical power | 0.8 | 0.482 |
| Total n | 53 | 26 |
| Degrees of freedom | 2 | 2 |
| Non-centrality parameter | 9.635 | 4.745 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.518 |
| Liver | | |
| Statistical power | 0.8 | 0.413 |
| Total n | 63 | 26 |
| Degrees of freedom | 2 | 2 |
| Non-centrality parameter | 9.635 | 3.979 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.587 |
| Paw | | |
| Statistical power | 0.8 | 0.115 |
| Total n | 313 | 26 |
| Degrees of freedom | 2 | 2 |
| Non-centrality parameter | 9.635 | 0.802 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.885 |
| Spleen | | |
| Statistical power | 0.8 | 0.6 |
| Total n | 129 | 83 |
| Degrees of freedom | 2 | 2 |

| | | |
|--------------------------|-------|-------|
| Non-centrality parameter | 9.635 | 6.219 |
| Type I error rate | 0.05 | 0.05 |
| Type II error rate | 0.2 | 0.4 |

The problem of the small and unequal sample sizes between groups became evident when we looked at the contingency tables for each tissue (Appendix table 115). Small counts in most cells made these analyses not very robust. However, looking at the contingency tables it can be observed that malnourished, sand fly inoculated mice experienced more frequently dissemination of parasites to other tissues than well-nourished, sand fly inoculated or malnourished, needle inoculated mice. The latter had generally the lowest frequency of dissemination events, suggesting that a) parasite inoculation by sand fly bite increased the frequency of parasite dissemination, and that b) in the context of sand fly inoculation, malnourishment further exacerbate the frequency of parasite dissemination.

Appendix Table 115
Odds Ratios

| Dissemination | MN_Needle | MN_SF | WN_SF |
|---------------|-----------|-----------|-----------|
| Brain | | | |
| NO | 3 | 4 | 5 |
| YES | 4 | 7 | 3 |
| Ear | | | |
| NO | 3 | 3 | 2 |
| YES | 8 | 12 | 10 |
| Eye | | | |
| NO | 7 | 8 | 4 |
| YES | 0 | 3 | 4 |
| Liver | | | |
| NO | 7 | 7 | 7 |
| YES | 0 | 4 | 1 |
| Paw | | | |
| NO | 6 | 8 | 7 |
| YES | 1 | 3 | 1 |
| Spleen | | | |
| NO | 12 | 13 | 20 |
| YES | 5 | 21 | 12 |

Logistic regression

We applied a logistic regression model to the same data and assessed the two predictor variables “Diet” and “Route” without an interaction term to assess individual predictor contribution to the outcome. The data output showed that infection by sand fly bite (“Route”) was statistically significant compared to needle inoculation only for the spleen. Malnourishment was close to being statistically significantly different from well-nourishment for the spleen, too. For all other tissues, there was no statistical significance observed (Appendix table 116).

Appendix Table 116
Logistic regression output

| Groups | Estimate | lower CI | upper CI | Std. Error | partial.R2 | z value | Pr(> z) | sig. |
|-------------|----------|-----------|----------|------------|------------|---------|----------|------|
| Brain | | | | | | | | |
| (Intercept) | -0.7828 | -3.2570 | 1.6385 | 1.2286 | 0.0000 | -0.6371 | 0.5241 | ns |
| DietMN | 1.0704 | -0.7742 | 3.0761 | 0.9624 | 0.0357 | 1.1123 | 0.2660 | ns |
| RouteSF | 0.2719 | -1.7200 | 2.2484 | 0.9880 | 0.0022 | 0.2752 | 0.7831 | ns |
| Ear | | | | | | | | |
| (Intercept) | 1.2040 | -1.1368 | 3.7552 | 1.2145 | 0.0000 | 0.9913 | 0.3215 | ns |
| DietMN | -0.2231 | -2.3937 | 1.7544 | 1.0083 | 0.0013 | -0.2213 | 0.8249 | ns |
| RouteSF | 0.4055 | -1.4841 | 2.3056 | 0.9354 | 0.0048 | 0.4335 | 0.6647 | ns |
| Eye | | | | | | | | |
| (Intercept) | -17.5852 | NA | 208.3362 | 2465.3259 | 0.0000 | -0.0071 | 0.9943 | ns |
| DietMN | -0.9808 | -3.0079 | 0.9169 | 0.9789 | 0.0411 | -1.0019 | 0.3164 | ns |
| RouteSF | 17.5852 | -313.1974 | NA | 2465.3258 | 0.1219 | 0.0071 | 0.9943 | ns |
| Liver | | | | | | | | |
| (Intercept) | -20.9524 | NA | 412.3356 | 4064.6350 | 0.0000 | -0.0052 | 0.9959 | ns |
| DietMN | 1.3863 | -0.8113 | 4.4936 | 1.2392 | 0.0663 | 1.1187 | 0.2633 | ns |
| RouteSF | 19.0065 | -457.9217 | NA | 4064.6349 | 0.1852 | 0.0047 | 0.9963 | ns |
| Paw | | | | | | | | |
| (Intercept) | -2.7568 | -6.6469 | 0.2147 | 1.6637 | 0.0000 | -1.6571 | 0.0975 | + |
| DietMN | 0.9651 | -1.3351 | 4.0962 | 1.2654 | 0.0252 | 0.7627 | 0.4457 | ns |
| RouteSF | 0.8109 | -1.5137 | 3.9522 | 1.2748 | 0.0174 | 0.6361 | 0.5247 | ns |
| Spleen | | | | | | | | |
| (Intercept) | -1.8659 | -3.3704 | -0.4616 | 0.7357 | 0.0000 | -2.5363 | 0.0112 | * |
| DietMN | 0.9904 | 0.0101 | 2.0112 | 0.5078 | 0.0350 | 1.9503 | 0.0511 | + |
| RouteSF | 1.3550 | 0.1458 | 2.6835 | 0.6387 | 0.0429 | 2.1217 | 0.0339 | * |

The odds ratios were nonsensical for eye and liver here (Appendix table 117), just as they had been for the contingency table analysis above (Appendix table 113). Looking at the spleen, the infection route had a bigger impact on parasite dissemination than diet, although either odds ratio was statistically significant according to the 95% confidence intervals. For all other tissues, we did not obtain statistical significance.

Appendix Table 117

Odds Ratios

| Groups | OR | 2.5 % | 97.5 % | Effect_size |
|-------------|----------|-------|-----------|-------------|
| Brain | | | | |
| (Intercept) | 0.457 | 0.038 | 5.147 | small |
| DietMN | 2.917 | 0.461 | 21.675 | small |
| RouteSF | 1.312 | 0.179 | 9.473 | very small |
| Ear | | | | |
| (Intercept) | 3.333 | 0.321 | 42.743 | small |
| DietMN | 0.800 | 0.091 | 5.780 | very small |
| RouteSF | 1.500 | 0.227 | 10.031 | very small |
| Eye | | | | |
| (Intercept) | 0.000 | NA | 3.01e+90 | large |
| DietMN | 0.375 | 0.049 | 2.502 | small |
| RouteSF | 4.34e+07 | 0.000 | NA | large |
| Liver | | | | |
| (Intercept) | 0.000 | NA | 1.19e+179 | large |
| DietMN | 4.000 | 0.444 | 89.442 | medium |
| RouteSF | 1.80e+08 | 0.000 | NA | large |
| Paw | | | | |
| (Intercept) | 0.064 | 0.001 | 1.240 | large |
| DietMN | 2.625 | 0.263 | 60.113 | small |
| RouteSF | 2.250 | 0.220 | 52.048 | small |
| Spleen | | | | |
| (Intercept) | 0.155 | 0.034 | 0.630 | medium |
| DietMN | 2.692 | 1.010 | 7.472 | small |
| RouteSF | 3.877 | 1.157 | 14.636 | medium |

The Wald test confirmed the logistic regression result, stating that only the infection route was statistically significant for the spleen, with “Diet” close to reaching statistical significance (Appendix table 118).

Appendix Table 118**Wald test**

| Predictor | chi2 | df | P | sig. |
|-----------|---------|----|--------|------|
| Brain | | | | |
| Diet | 1.2372 | 1 | 0.2660 | ns |
| Route | 0.0758 | 1 | 0.7831 | ns |
| Ear | | | | |
| Diet | 0.0490 | 1 | 0.8249 | ns |
| Route | 0.1879 | 1 | 0.6647 | ns |
| Eye | | | | |
| Diet | 1.0039 | 1 | 0.3164 | ns |
| Route | <0.0001 | 1 | 0.9943 | ns |
| Liver | | | | |
| Diet | 1.2514 | 1 | 0.2633 | ns |
| Route | <0.0001 | 1 | 0.9963 | ns |
| Paw | | | | |
| Diet | 0.5817 | 1 | 0.4457 | ns |
| Route | 0.4047 | 1 | 0.5247 | ns |
| Spleen | | | | |
| Diet | 3.8037 | 1 | 0.0511 | + |
| Route | 4.5017 | 1 | 0.0339 | * |

However, although, in general, there was no statistically significance in the logistic regression model with the exception of the infection route in the spleen that did not mean that there was no meaningful biological effect. A retrospective sample size and power calculation with the study data showed that the study was well underpowered for the logistic regression for all tissues (Appendix table 119), as it had already been the case for the contingency table analysis (Appendix table 114), suggesting that the lack of statistical significance may be due to type II errors. But larger sample sizes as indicated by the sample size calculation, were prohibitive due to cost and loss of life.

Appendix Table 119**Retrospective power analyses**

| Predictor | Calculation | Beta0 | Beta1 | R-square | alpha | Power | TotalN | NCP | Alternative |
|-----------|-------------|-------|-------|----------|-------|-------|--------|-----|-------------|
| Brain | | | | | | | | | |

| | | | | | | | | | |
|--------|-------------|---------|--------|-------|------|-------------|----------------|--------|-----------|
| Diet | Sample_size | 0.154 | 1.070 | 0.002 | 0.05 | 0.80 | 130 | 2.769 | not equal |
| Route | Sample_size | 0.154 | 0.272 | 0.036 | 0.05 | 0.80 | 1805 | 2.800 | not equal |
| Diet | Power | -0.783 | 1.070 | 0.002 | 0.05 | 0.25 | 26 | 1.305 | not equal |
| Route | Power | -0.783 | 0.272 | 0.036 | 0.05 | 0.06 | 26 | 0.323 | not equal |
| Ear | | | | | | | | | |
| Diet | Sample_size | 1.322 | -0.223 | 0.005 | 0.05 | 0.80 | 3590 | -2.799 | not equal |
| Route | Sample_size | 1.322 | 0.405 | 0.001 | 0.05 | 0.80 | 1314 | 2.793 | not equal |
| Diet | Power | 1.204 | -0.223 | 0.005 | 0.05 | 0.06 | 38 | -0.297 | not equal |
| Route | Power | 1.204 | 0.405 | 0.001 | 0.05 | 0.08 | 38 | 0.493 | not equal |
| Eye | | | | | | | | | |
| Diet | Sample_size | -0.999 | -0.981 | 0.122 | 0.05 | 0.80 | 260 | -2.758 | not equal |
| Route | Sample_size | -0.999 | 17.585 | 0.041 | 0.05 | 0.80 | 563119 | 2.286 | not equal |
| Diet | Power | -17.585 | -0.981 | 0.122 | 0.05 | 0.03 | 26 | 0.000 | not equal |
| Route | Power | -17.585 | 17.585 | 0.041 | 0.05 | 0.00 | 26 | 0.009 | not equal |
| Liver | | | | | | | | | |
| Diet | Sample_size | -1.435 | 1.386 | 0.185 | 0.05 | 0.80 | 101 | 2.747 | not equal |
| Route | Sample_size | -1.435 | 19.006 | 0.066 | 0.05 | 0.80 | 1325232 | 2.286 | not equal |
| Diet | Power | -20.952 | 1.386 | 0.185 | 0.05 | 0.02 | 26 | 0.000 | not equal |
| Route | Power | -20.952 | 19.006 | 0.066 | 0.05 | 0.00 | 26 | 0.002 | not equal |
| Paw | | | | | | | | | |
| Diet | Sample_size | -1.435 | 0.965 | 0.017 | 0.05 | 0.80 | 179 | 2.770 | not equal |
| Route | Sample_size | -1.435 | 0.811 | 0.025 | 0.05 | 0.80 | 261 | 2.778 | not equal |
| Diet | Power | -2.757 | 0.965 | 0.017 | 0.05 | 0.09 | 26 | 0.677 | not equal |
| Route | Power | -2.757 | 0.811 | 0.025 | 0.05 | 0.07 | 26 | 0.556 | not equal |
| Spleen | | | | | | | | | |
| Diet | Sample_size | -0.169 | 0.990 | 0.043 | 0.05 | 0.80 | 144 | 2.779 | not equal |
| Route | Sample_size | -0.169 | 1.355 | 0.035 | 0.05 | 0.80 | 82 | 2.756 | not equal |
| Diet | Power | -1.866 | 0.990 | 0.043 | 0.05 | 0.39 | 83 | 1.703 | not equal |
| Route | Power | -1.866 | 1.355 | 0.035 | 0.05 | 0.68 | 83 | 2.389 | not equal |

Even so, there was a good indication of potential biological significance. Considering the predicted probability of parasite dissemination for most tissues, it can be seen that being malnourished and inoculated by a sand fly increased the probability of parasite dissemination for most tissues beyond the other conditions (Appendix table 120). With exception of the spleen, the large confidence intervals did not render statistical significance in all other tissues, which did not exclude biological significance, though.

Appendix Table 120
Predicted probability of parasite dissemination

| Factors | Diet | Route | fit | se.fit | Predicted_Probability | lower_CI | upper_CI |
|---------|------|--------|----------|-----------|-----------------------|----------|----------|
| Brain | | | | | | | |
| 1 | WN | SF | -0.5108 | 0.7303 | 0.3750 | 0.1254 | 0.7152 |
| 2 | MN | Needle | 0.2877 | 0.7638 | 0.5714 | 0.2298 | 0.8563 |
| 3 | MN | SF | 0.5596 | 0.6268 | 0.6364 | 0.3387 | 0.8567 |
| Ear | | | | | | | |
| 1 | WN | SF | 1.6094 | 0.7746 | 0.8333 | 0.5228 | 0.9580 |
| 2 | MN | Needle | 0.9808 | 0.6770 | 0.7273 | 0.4143 | 0.9095 |
| 3 | MN | SF | 1.3863 | 0.6455 | 0.8000 | 0.5302 | 0.9341 |
| Eye | | | | | | | |
| 1 | WN | SF | 0.0000 | 0.7071 | 0.5000 | 0.2001 | 0.7999 |
| 2 | MN | Needle | -18.5661 | 2465.3257 | 0.0000 | 0.0000 | 1.0000 |
| 3 | MN | SF | -0.9808 | 0.6770 | 0.2727 | 0.0905 | 0.5857 |
| Liver | | | | | | | |
| 1 | WN | SF | -1.9459 | 1.0690 | 0.1250 | 0.0173 | 0.5373 |
| 2 | MN | Needle | -19.5661 | 4064.6348 | 0.0000 | 0.0000 | 1.0000 |
| 3 | MN | SF | -0.5596 | 0.6268 | 0.3636 | 0.1433 | 0.6613 |
| Paw | | | | | | | |
| 1 | WN | SF | -1.9459 | 1.0690 | 0.1250 | 0.0173 | 0.5373 |
| 2 | MN | Needle | -1.7918 | 1.0801 | 0.1429 | 0.0197 | 0.5806 |
| 3 | MN | SF | -0.9808 | 0.6770 | 0.2727 | 0.0905 | 0.5857 |
| Spleen | | | | | | | |
| 1 | WN | SF | -0.5108 | 0.3651 | 0.3750 | 0.2268 | 0.5510 |
| 2 | MN | Needle | -0.8755 | 0.5323 | 0.2941 | 0.1280 | 0.5419 |
| 3 | MN | SF | 0.4796 | 0.3529 | 0.6176 | 0.4472 | 0.7634 |

Figure 4

Panel a

Here, we analyzed the proportion of necrophiliacs, monocytes and lymphocytes in terminal bleeds from in well-nourished (WN) and malnourished (MN) BALB/c mice infected with *Leishmania donovani* parasite via sand fly bites (SF) for up to 30 weeks or not. We analyzed a total of $N=40$ BALB/c mice. The different groups had varying sample sizes (Appendix table 121). For the data analysis, we tested Poisson and

negative binomial regression models of the normalized cell counts, or beta regression after conversion of percentiles to ratios. Based on the Akaike information criterion (AIC), we selected a beta_regression model for the data analysis post data conversion. The model fit of the data was reasonable producing no statistically significant departure from 1 for its dispersion ratio and producing reasonable pseudo-R² values of all three cell groups (Appendix table 121).

Appendix Table 121
Summary information

| Cells | Total N | MN_Ctrl | WN_Ctrl | MN_SF | WN_SF | Dispersion Ratio | Pseudo R ² |
|-------------|---------|---------|---------|-------|-------|------------------|-----------------------|
| Lymphocytes | 40 | 12 | 10 | 10 | 8 | 1.1012 | 0.5751 |
| Monocytes | 40 | 12 | 10 | 10 | 8 | 1.2556 | 0.5175 |
| Neutrophils | 40 | 12 | 10 | 10 | 8 | 1.1267 | 0.5146 |
| Other | 40 | 12 | 10 | 10 | 8 | 1.2062 | 0.1610 |

The model output showed that infection “Route” was always a statistically significant predictor for all three cell types, while the nutritional state of the mice had a statistical significant effect only for monocytes (Appendix table 122). The interaction between “Route” and “Diet” was statistically significant for lymphocytes and monocytes, but not for neutrophils. Please, note that “other” referred to the small reminder of detected blood cells that were not accounted for.

Appendix Table 122
Beta regression model output

| Factors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|--------------------|----------|------------|----------|----------|------|
| Lymphocytes | | | | | |
| (Intercept) | 0.8708 | 0.1195 | 7.2865 | <0.0001 | **** |
| DietWN | 0.2216 | 0.1814 | 1.2216 | 0.2219 | ns |
| RouteSF | -0.9575 | 0.1695 | -5.6489 | <0.0001 | **** |
| DietWN:RouteSF | 0.7197 | 0.2618 | 2.7486 | 0.0060 | ** |
| Monocytes | | | | | |
| (Intercept) | -5.0620 | 0.2985 | -16.9568 | <0.0001 | **** |
| DietWN | 1.2948 | 0.3442 | 3.7624 | 0.0002 | *** |
| RouteSF | 2.1254 | 0.3241 | 6.5577 | <0.0001 | **** |
| DietWN:RouteSF | -1.9161 | 0.4384 | -4.3709 | <0.0001 | **** |
| Neutrophils | | | | | |
| (Intercept) | -1.0850 | 0.1289 | -8.4149 | <0.0001 | **** |
| DietWN | -0.3793 | 0.2010 | -1.8871 | 0.0591 | + |
| RouteSF | 0.7821 | 0.1796 | 4.3559 | <0.0001 | **** |

| | | | | | |
|----------------|---------|--------|----------|---------|------|
| DietWN:RouteSF | -0.3838 | 0.2838 | -1.3520 | 0.1764 | ns |
| Other | | | | | |
| (Intercept) | -3.1699 | 0.1470 | -21.5603 | <0.0001 | **** |
| DietWN | 0.0428 | 0.2119 | 0.2020 | 0.8399 | ns |
| RouteSF | -0.2999 | 0.2288 | -1.3108 | 0.1899 | ns |
| DietWN:RouteSF | -0.2699 | 0.3507 | -0.7696 | 0.4415 | ns |

The likelihood ratio test confirmed that the inclusion of both predictors in the model was statistically significant for all three cell types (Appendix table 123).

Appendix Table 123
Likelihood ratio test against null model

| #Df | LogLik | Df | Chisq | Pr(>Chisq) | sig. |
|-------------|----------|----|---------|------------|------|
| Lymphocytes | | | | | |
| 5 | 41.0899 | NA | NA | NA | ? |
| 2 | 24.5001 | -3 | 33.1796 | <0.0001 | **** |
| Monocytes | | | | | |
| 5 | 125.8189 | NA | NA | NA | ? |
| 2 | 107.8980 | -3 | 35.8418 | <0.0001 | **** |
| Neutrophils | | | | | |
| 5 | 42.3118 | NA | NA | NA | ? |
| 2 | 28.9977 | -3 | 26.6283 | <0.0001 | **** |
| Other | | | | | |
| 5 | 106.1766 | NA | NA | NA | ? |
| 2 | 103.0245 | -3 | 6.3041 | 0.0977 | + |

The pairwise comparison was based on the estimated marginal means. For lymphocytes, it showed that malnourished, sand fly inoculated mice had statistically significantly less lymphocytes in the blood than the three other groups (Appendix table 124 & Appendix table 125). For monocytes, the pairwise comparison showed that malnourished control animals had statistically significantly less monocytes in circulation. Conversely, after infection by sand fly bite, malnourished mice had statistically significantly more monocytes in circulation. For neutrophils, we observed statistically significantly more neutrophils in circulation in malnourished, sand fly inoculated mice compared to the other three groups.

Appendix Table 124
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-------------------|----------|--------|-----|---------|---------|------|
| Lymphocytes | | | | | | |
| MN Ctrl - WN Ctrl | -0.0439 | 0.0357 | Inf | -1.2295 | 0.2827 | ns |
| MN Ctrl - MN SF | 0.2266 | 0.0390 | Inf | 5.8162 | <0.0001 | **** |
| WN Ctrl - WN SF | 0.0473 | 0.0399 | Inf | 1.1862 | 0.2827 | ns |
| MN SF - WN SF | -0.2232 | 0.0428 | Inf | -5.2151 | <0.0001 | **** |
| Monocytes | | | | | | |
| MN Ctrl - WN Ctrl | -0.0163 | 0.0049 | Inf | -3.3427 | 0.0017 | ** |
| MN Ctrl - MN SF | -0.0441 | 0.0075 | Inf | -5.8959 | <0.0001 | **** |
| WN Ctrl - WN SF | -0.0051 | 0.0074 | Inf | -0.6920 | 0.4890 | ns |
| MN SF - WN SF | 0.0227 | 0.0093 | Inf | 2.4337 | 0.0179 | * |
| Neutrophils | | | | | | |
| MN Ctrl - WN Ctrl | 0.0648 | 0.0338 | Inf | 1.9150 | 0.0832 | + |
| MN Ctrl - MN SF | -0.1723 | 0.0391 | Inf | -4.4110 | <0.0001 | **** |
| WN Ctrl - WN SF | -0.0684 | 0.0380 | Inf | -1.7977 | 0.0867 | + |
| MN SF - WN SF | 0.1687 | 0.0428 | Inf | 3.9445 | 0.0002 | *** |
| Other | | | | | | |
| MN Ctrl - WN Ctrl | -0.0017 | 0.0084 | Inf | -0.2016 | 0.8402 | ns |
| MN Ctrl - MN SF | 0.0101 | 0.0076 | Inf | 1.3292 | 0.2757 | ns |
| WN Ctrl - WN SF | 0.0178 | 0.0081 | Inf | 2.2079 | 0.0988 | + |
| MN SF - WN SF | 0.0060 | 0.0073 | Inf | 0.8227 | 0.4928 | ns |

Appendix Table 125
Pairwise comparison letter code

| Diet | Route | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|-------------|-------|--------|--------|-----|------------|------------|--------|
| Lymphocytes | | | | | | | |
| MN | SF | 0.4783 | 0.0300 | Inf | 0.4034 | 0.5532 | a |
| WN | SF | 0.7015 | 0.0305 | Inf | 0.6253 | 0.7778 | b |
| MN | Ctrl | 0.7049 | 0.0249 | Inf | 0.6428 | 0.7670 | b |
| WN | Ctrl | 0.7488 | 0.0258 | Inf | 0.6844 | 0.8132 | b |
| Monocytes | | | | | | | |
| MN | Ctrl | 0.0063 | 0.0019 | Inf | 0.0016 | 0.0110 | a |

| | | | | | | | |
|-------------|------|--------|--------|-----|--------|--------|---|
| WN | Ctrl | 0.0226 | 0.0048 | Inf | 0.0107 | 0.0345 | b |
| WN | SF | 0.0277 | 0.0060 | Inf | 0.0128 | 0.0426 | b |
| MN | SF | 0.0504 | 0.0074 | Inf | 0.0319 | 0.0689 | c |
| Neutrophils | | | | | | | |
| WN | Ctrl | 0.1878 | 0.0237 | Inf | 0.1286 | 0.2471 | a |
| MN | Ctrl | 0.2526 | 0.0243 | Inf | 0.1918 | 0.3134 | a |
| WN | SF | 0.2562 | 0.0299 | Inf | 0.1814 | 0.3309 | a |
| MN | SF | 0.4249 | 0.0306 | Inf | 0.3484 | 0.5013 | b |
| Other | | | | | | | |
| WN | SF | 0.0242 | 0.0052 | Inf | 0.0112 | 0.0372 | a |
| MN | SF | 0.0302 | 0.0053 | Inf | 0.0169 | 0.0434 | a |
| MN | Ctrl | 0.0403 | 0.0057 | Inf | 0.0261 | 0.0545 | a |
| WN | Ctrl | 0.0420 | 0.0063 | Inf | 0.0261 | 0.0579 | a |

Panel b

Data analysis

Here, we present the serum chemistry analysis of a total of $N=35$ well-nourished (WN) or malnourished (MN) BALB/c mice infected by sand fly bite (SF) or not (Ctrl) (Appendix table 126). A total of 8 targets were measured (alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), Globulin (GLOB), Glucose (GLU), total protein (TP) & urea nitrogen (BUN)) per mouse.

Appendix Table 126
Summary information

| Targets | Total N | WN_Ctrl | MN_Ctrl | WN_SF | MN_SF |
|--------------------------------|---------|---------|---------|-------|-------|
| alanine aminotransferase (ALT) | 35 | 7 | 6 | 10 | 12 |
| albumin (ALB) | 35 | 7 | 6 | 10 | 12 |
| alkaline phosphatase (ALP) | 35 | 7 | 6 | 10 | 12 |
| amylase (AMY) | 35 | 7 | 6 | 10 | 12 |
| Globulin (GLOB) | 35 | 7 | 6 | 10 | 12 |
| Glucose (GLU) | 35 | 7 | 6 | 10 | 12 |
| total protein (TP) | 35 | 7 | 6 | 10 | 12 |
| urea nitrogen (BUN) | 35 | 7 | 6 | 10 | 12 |

Appendix Table 126
Summary information (continued)

| Targets | Transformation |
|--------------------------------|---------------------------|
| alanine aminotransferase (ALT) | Box-Cox power Transformed |
| albumin (ALB) | untransformed |
| alkaline phosphatase (ALP) | Box-Cox power Transformed |
| amylase (AMY) | Box-Cox power Transformed |
| Globulin (GLOB) | Box-Cox power Transformed |
| Glucose (GLU) | untransformed |
| total protein (TP) | untransformed |
| urea nitrogen (BUN) | untransformed |

We needed to analyze the data with a two-way approach to account for the two predictors, “Diet” and “Route”, both of which were between-subject factors.

For a two-way ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance
- No significant outliers

Assumption analyses

Data normality

The assessment of the untransformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by both predictors. Note that all groups consisted of <30 data points (Appendix table 126), which made groups too small to assess data distribution reliably by Shapiro-Wilks test. We executed the test anyway as an indicator of gross departure of data normality. Thus, we performed the analyses by Shapiro-Wilks test (Appendix table 127) and QQ-plots (Fig.S1a-1). We found occasional departure of normality, which could not all be remedied by data transformation.

Appendix Table 127
Univariate Shapiro-Wilks test results

| Diet | Route | variable | statistic | p | Outcome |
|--------------------------------|-------|----------|-----------|--------|---------|
| alanine aminotransferase (ALT) | | | | | |
| WN | Ctrl | Counts | 0.7818 | 0.0269 | sig. |
| WN | SF | Counts | 0.9528 | 0.7013 | ns |
| MN | Ctrl | Counts | 0.8360 | 0.1207 | ns |
| MN | SF | Counts | 0.8928 | 0.1280 | ns |
| albumin (ALB) | | | | | |
| WN | Ctrl | Counts | 0.9666 | 0.8733 | ns |

| | | | | | |
|----------------------------|------|--------|--------|--------|------|
| WN | SF | Counts | 0.9584 | 0.7680 | ns |
| MN | Ctrl | Counts | 0.9380 | 0.6433 | ns |
| MN | SF | Counts | 0.9607 | 0.7937 | ns |
| alkaline phosphatase (ALP) | | | | | |
| WN | Ctrl | Counts | 0.9451 | 0.6854 | ns |
| WN | SF | Counts | 0.8895 | 0.1673 | ns |
| MN | Ctrl | Counts | 0.8065 | 0.0672 | ns |
| MN | SF | Counts | 0.9450 | 0.5652 | ns |
| amylase (AMY) | | | | | |
| WN | Ctrl | Counts | 0.9726 | 0.9167 | ns |
| WN | SF | Counts | 0.9436 | 0.5932 | ns |
| MN | Ctrl | Counts | 0.8452 | 0.1438 | ns |
| MN | SF | Counts | 0.8068 | 0.0112 | sig. |
| Globulin (GLOB) | | | | | |
| WN | Ctrl | Counts | 0.6004 | 0.0003 | sig. |
| WN | SF | Counts | 0.7753 | 0.0073 | sig. |
| MN | Ctrl | Counts | 0.7315 | 0.0130 | sig. |
| MN | SF | Counts | 0.8712 | 0.0677 | ns |
| Glucose (GLU) | | | | | |
| WN | Ctrl | Counts | 0.8510 | 0.1256 | ns |
| WN | SF | Counts | 0.9041 | 0.2431 | ns |
| MN | Ctrl | Counts | 0.9287 | 0.5699 | ns |
| MN | SF | Counts | 0.8833 | 0.0966 | ns |
| total protein (TP) | | | | | |
| WN | Ctrl | Counts | 0.8936 | 0.2939 | ns |
| WN | SF | Counts | 0.9169 | 0.3319 | ns |
| MN | Ctrl | Counts | 0.9999 | 1.0000 | ns |
| MN | SF | Counts | 0.9164 | 0.2578 | ns |
| urea nitrogen (BUN) | | | | | |
| WN | Ctrl | Counts | 0.9630 | 0.8441 | ns |
| WN | SF | Counts | 0.9304 | 0.4519 | ns |
| MN | Ctrl | Counts | 0.9182 | 0.4928 | ns |
| MN | SF | Counts | 0.8974 | 0.1469 | ns |

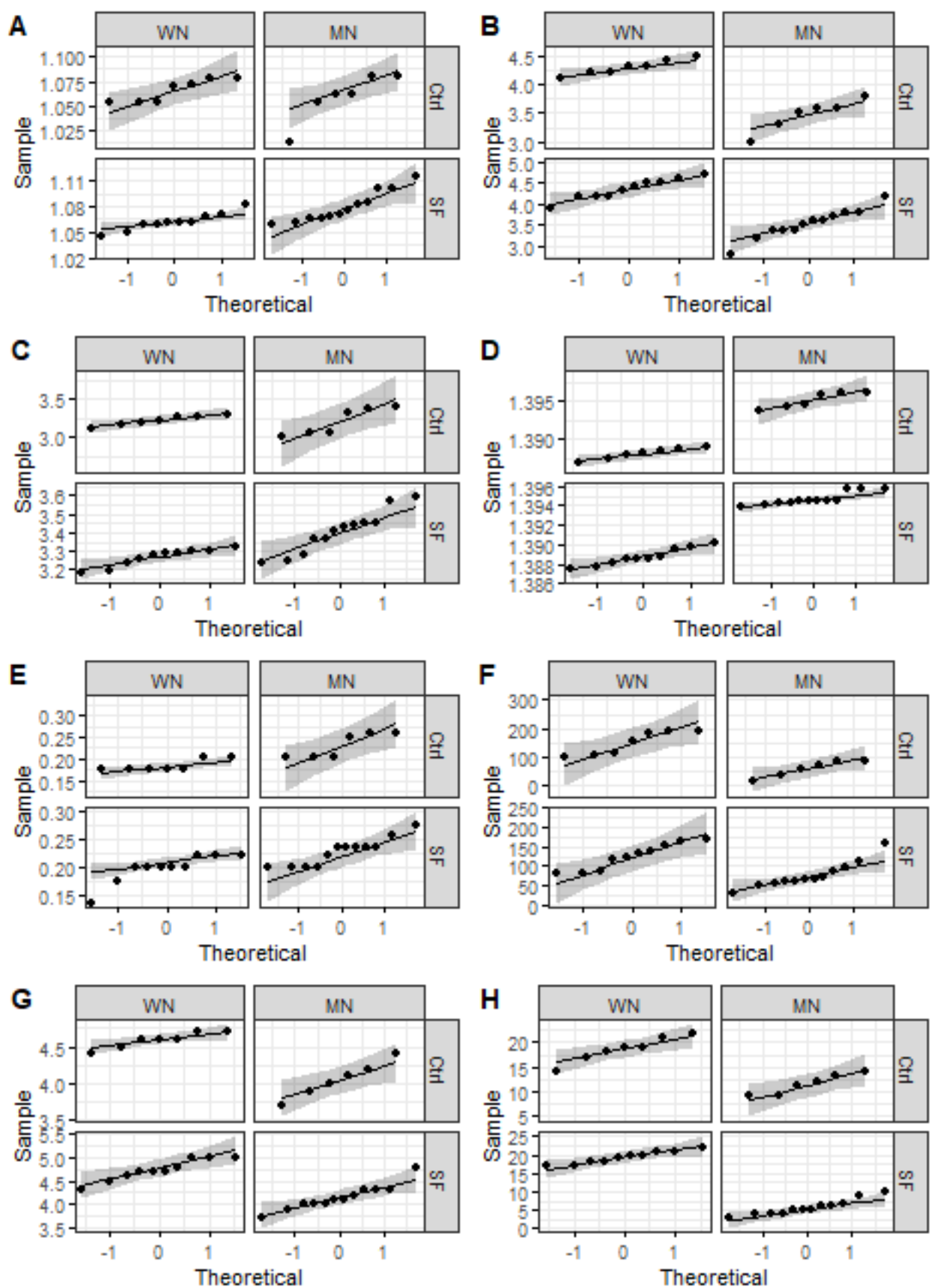


Fig.4b-1: QQ-plots of serum chemistry data: A) alanine aminotransferase (ALT), B) albumin (ALB), C) alkaline phosphatase (ALP), D) amylase (AMY), E) Globulin (GLOB), F) Glucose (GLU), G) total protein (TP) and H) urea nitrogen (BUN)

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset. The analysis output showed that assumption of homogeneity between groups held all targets except for alkaline phosphatase (Appendix table 128).

Appendix Table 128
Assessment of homogeneity of variance by target

| Targets | df1 | df2 | statistic | p | sig. |
|--------------------------------|-----|-----|-----------|--------|------|
| alanine aminotransferase (ALT) | 3 | 31 | 1.1073 | 0.3610 | ns |
| albumin (ALB) | 3 | 31 | 1.0760 | 0.3736 | ns |
| alkaline phosphatase (ALP) | 3 | 31 | 8.5139 | 0.0003 | *** |
| amylase (AMY) | 3 | 31 | 1.2940 | 0.2939 | ns |
| Globulin (GLOB) | 3 | 31 | 1.4187 | 0.2560 | ns |
| Glucose (GLU) | 3 | 31 | 0.6033 | 0.6178 | ns |
| total protein (TP) | 3 | 31 | 0.9974 | 0.4070 | ns |
| urea nitrogen (BUN) | 3 | 31 | 0.1293 | 0.9419 | ns |

Outliers

Conversely, we detected outliers for all targets except for alkaline phosphatase (Appendix table 129). Only one of these outliers was classed as extreme (Globulin (GLOB)).

Appendix Table 129
List of possible outliers

| Diet | Route | Tissue | is.outlier | is.extreme |
|--------------------------------|-------|--------------------------------|------------|------------|
| alanine aminotransferase (ALT) | | | | |
| MN | Ctrl | alanine aminotransferase (ALT) | TRUE | FALSE |
| WN | SF | alanine aminotransferase (ALT) | TRUE | FALSE |
| WN | SF | alanine aminotransferase (ALT) | TRUE | FALSE |
| albumin (ALB) | | | | |
| MN | SF | albumin (ALB) | TRUE | FALSE |
| amylase (AMY) | | | | |
| MN | SF | amylase (AMY) | TRUE | FALSE |
| MN | SF | amylase (AMY) | TRUE | FALSE |
| Globulin (GLOB) | | | | |
| WN | SF | Globulin (GLOB) | TRUE | FALSE |

| | | | | |
|---------------------|----|---------------------|------|-------|
| WN | SF | Globulin (GLOB) | TRUE | TRUE |
| Glucose (GLU) | | | | |
| MN | SF | Glucose (GLU) | TRUE | FALSE |
| total protein (TP) | | | | |
| MN | SF | total protein (TP) | TRUE | FALSE |
| urea nitrogen (BUN) | | | | |
| MN | SF | urea nitrogen (BUN) | TRUE | FALSE |

Two-way analysis

Based on the assumption analysis, we applied appropriate two-way tests for each target (Appendix table 130).

Appendix Table 130
Applied tests

| Targets | Two-way test | One-way test |
|--------------------------------|--------------------------|--------------------------------------|
| alanine aminotransferase (ALT) | Robust two-way ANOVA | Robust one-way ANOVA |
| albumin (ALB) | Standard two-way ANOVA | Standard one-way ANOVA |
| alkaline phosphatase (ALP) | Robust two-way ANOVA | Robust one-way ANOVA |
| amylase (AMY) | Robust two-way ANOVA | Robust one-way ANOVA |
| Globulin (GLOB) | Simple linear regression | Not applicable for linear regression |
| Glucose (GLU) | Standard two-way ANOVA | Standard one-way ANOVA |
| total protein (TP) | Standard two-way ANOVA | Standard one-way ANOVA |
| urea nitrogen (BUN) | Standard two-way ANOVA | Standard one-way ANOVA |

Appendix Table 130
Applied tests (continued)

| Targets | Pairwise |
|--------------------------------|-----------------------------------|
| alanine aminotransferase (ALT) | Linear contrast expression |
| albumin (ALB) | Estimated marginal means analysis |
| alkaline phosphatase (ALP) | Linear contrast expression |
| amylase (AMY) | Linear contrast expression |
| Globulin (GLOB) | Estimated marginal means analysis |
| Glucose (GLU) | Estimated marginal means analysis |
| total protein (TP) | Estimated marginal means analysis |
| urea nitrogen (BUN) | Estimated marginal means analysis |

The respective two-way analyses showed that there was no statistical significant differences detected for alanine aminotransferase and alkaline phosphatase (Appendix table 131). All other targets had detectable statistical significant differences for “Diet”, suggesting that any detected significant difference detected in target levels were due to the nutritional status of the mice rather than the infection “Route”. Only urea nitrogen showed statistical significance for “Route” and the interaction term, suggesting that both predictor were relevant to the detected levels of this target.

Appendix Table 131
Two-way analysis

| Effect | DFn | DFd | Statistic | p.value | p<.05 | ges |
|--------------------------------|-----|-----|-----------|---------|-------|-------|
| alanine aminotransferase (ALT) | | | | | | |
| Diet | 1 | 31 | 1.2224 | 0.2910 | | NA |
| Route | 1 | 31 | 0.3431 | 0.5690 | | NA |
| Diet:Route | 1 | 31 | 1.6730 | 0.2200 | | NA |
| albumin (ALB) | | | | | | |
| Diet | 1 | 31 | 77.1180 | <0.0001 | * | 0.713 |
| Route | 1 | 31 | 0.4620 | 0.5020 | | 0.015 |
| Diet:Route | 1 | 31 | 0.0002 | 0.9900 | | 0.000 |
| alkaline phosphatase (ALP) | | | | | | |
| Diet | 1 | 31 | 0.7158 | 0.4390 | | NA |
| Route | 1 | 31 | 4.3699 | 0.0860 | + | NA |
| Diet:Route | 1 | 31 | 1.5655 | 0.2680 | | NA |
| amylase (AMY) | | | | | | |
| Diet | 1 | 31 | 288.2789 | 0.0010 | *** | NA |
| Route | 1 | 31 | 0.0104 | 0.9220 | | NA |
| Diet:Route | 1 | 31 | 1.8554 | 0.2040 | | NA |
| Globulin (GLOB) | | | | | | |
| Diet | 1 | 31 | 20.7636 | 0.0001 | **** | NA |
| Route | 1 | 31 | 1.0594 | 0.3113 | | NA |
| Diet:Route | 1 | 31 | 0.8660 | 0.3593 | | NA |
| Glucose (GLU) | | | | | | |
| Diet | 1 | 31 | 29.2640 | <0.0001 | * | 0.486 |
| Route | 1 | 31 | 0.0540 | 0.8170 | | 0.002 |
| Diet:Route | 1 | 31 | 3.2070 | 0.0830 | | 0.094 |
| total protein (TP) | | | | | | |

| | | | | | | |
|---------------------|---|----|----------|---------|---|-------|
| Diet | 1 | 31 | 52.0380 | <0.0001 | * | 0.627 |
| Route | 1 | 31 | 2.1060 | 0.1570 | | 0.064 |
| Diet:Route | 1 | 31 | 0.1040 | 0.7490 | | 0.003 |
| urea nitrogen (BUN) | | | | | | |
| Diet | 1 | 31 | 243.9170 | <0.0001 | * | 0.887 |
| Route | 1 | 31 | 10.5640 | 0.0030 | * | 0.254 |
| Diet:Route | 1 | 31 | 18.3990 | 0.0002 | * | 0.372 |

For the analysis of the simple main effect for each respective between-subject factor for each target, we performed one-way analyses with the data split by the predictor that was not used within the function. The results showed that statistical significance for each target, including those that had not shown statistical significance in the two-way analysis (Appendix table 132). Again all samples showed statistical significant differences for the comparison of well-nourished and malnourished mice for both control and sand fly infected ones. Only alkaline phosphatase and urea nitrogen also showed statistical significance for the comparison of control vs sand fly infected mice, but only for the malnourished mice.

Appendix Table 132
Simple main effect analysis

| Factor | Effect | statistic | df1 | df2 | p.value | Sig. | effsize |
|--------------------------------|--------|-----------|-----|---------|---------|------|---------|
| alanine aminotransferase (ALT) | | | | | | | |
| WN | Route | 0.8436 | 1 | 11.0300 | 0.3780 | ns | 0.3520 |
| MN | Route | 2.5191 | 1 | 7.9018 | 0.1516 | ns | 0.6778 |
| Ctrl | Diet | 0.4190 | 1 | 6.5740 | 0.5394 | ns | 0.3388 |
| SF | Diet | 6.6157 | 1 | 13.2865 | 0.0229 | * | 0.7166 |
| albumin (ALB) | | | | | | | |
| WN | Route | 0.2260 | 1 | 31.0000 | 0.6380 | ns | 0.0070 |
| MN | Route | 0.2360 | 1 | 31.0000 | 0.6300 | ns | 0.0080 |
| Ctrl | Diet | 28.7920 | 1 | 31.0000 | <0.0001 | * | 0.4820 |
| SF | Diet | 48.3270 | 1 | 31.0000 | <0.0001 | * | 0.6090 |
| alkaline phosphatase (ALP) | | | | | | | |
| WN | Route | 2.5948 | 1 | 11.5678 | 0.1341 | ns | 0.4663 |
| MN | Route | 5.8150 | 1 | 7.8255 | 0.0431 | * | 0.7513 |
| Ctrl | Diet | 0.0521 | 1 | 6.0890 | 0.8270 | ns | 0.2699 |
| SF | Diet | 9.7419 | 1 | 12.6641 | 0.0083 | ** | 0.8595 |
| amylase (AMY) | | | | | | | |
| WN | Route | 2.2199 | 1 | 12.8555 | 0.1604 | ns | 0.5173 |

| | | | | | | | |
|---------------------|-------|----------|---|---------|---------|------|--------|
| MN | Route | 0.5645 | 1 | 7.8712 | 0.4743 | ns | 0.2528 |
| Ctrl | Diet | 208.9552 | 1 | 8.7817 | <0.0001 | **** | 1.1061 |
| SF | Diet | 248.6190 | 1 | 12.8663 | <0.0001 | **** | 1.1373 |
| Glucose (GLU) | | | | | | | |
| WN | Route | 2.0250 | 1 | 31.0000 | 0.1650 | ns | 0.0610 |
| MN | Route | 1.2360 | 1 | 31.0000 | 0.2750 | ns | 0.0380 |
| Ctrl | Diet | 22.2650 | 1 | 31.0000 | <0.0001 | * | 0.4180 |
| SF | Diet | 10.2060 | 1 | 31.0000 | 0.0030 | * | 0.2480 |
| total protein (TP) | | | | | | | |
| WN | Route | 1.5880 | 1 | 31.0000 | 0.2170 | ns | 0.0490 |
| MN | Route | 0.6230 | 1 | 31.0000 | 0.4360 | ns | 0.0200 |
| Ctrl | Diet | 17.1730 | 1 | 31.0000 | 0.0002 | * | 0.3560 |
| SF | Diet | 34.9690 | 1 | 31.0000 | <0.0001 | * | 0.5300 |
| urea nitrogen (BUN) | | | | | | | |
| WN | Route | 0.4850 | 1 | 31.0000 | 0.4920 | ns | 0.0150 |
| MN | Route | 28.4790 | 1 | 31.0000 | <0.0001 | * | 0.4790 |
| Ctrl | Diet | 37.5290 | 1 | 31.0000 | <0.0001 | * | 0.5480 |
| SF | Diet | 224.7880 | 1 | 31.0000 | <0.0001 | * | 0.8790 |

Since both predictors had only two levels each, the pairwise comparison reflected the observations of the one-way analyses above (Appendix table 133).

Appendix Table 133
Pairwise comparison

| contrast | df | statistic | p.value | Sig. |
|--------------------------------|----|-----------|---------|------|
| alanine aminotransferase (ALT) | | | | |
| MN Ctrl - MN SF | 31 | -0.0188 | 0.1516 | ns |
| WN Ctrl - MN Ctrl | 31 | 0.0073 | 0.5394 | ns |
| WN Ctrl - WN SF | 31 | 0.0046 | 0.3780 | ns |
| WN SF - MN SF | 31 | -0.0162 | 0.0229 | * |
| albumin (ALB) | | | | |
| MN Ctrl - MN SF | 31 | -0.4860 | 0.6304 | ns |
| WN Ctrl - MN Ctrl | 31 | 5.3658 | <0.0001 | **** |
| WN Ctrl - WN SF | 31 | -0.4755 | 0.6378 | ns |
| WN SF - MN SF | 31 | 6.9518 | <0.0001 | **** |

alkaline phosphatase (ALP)

| | | | | |
|-------------------|----|---------|--------|----|
| MN Ctrl - MN SF | 31 | -0.1965 | 0.0431 | * |
| WN Ctrl - MN Ctrl | 31 | 0.0173 | 0.8270 | ns |
| WN Ctrl - WN SF | 31 | -0.0481 | 0.1341 | ns |
| WN SF - MN SF | 31 | -0.1312 | 0.0083 | ** |

amylase (AMY)

| | | | | |
|-------------------|----|---------|---------|------|
| MN Ctrl - MN SF | 31 | 0.0003 | 0.4743 | ns |
| WN Ctrl - MN Ctrl | 31 | -0.0070 | <0.0001 | **** |
| WN Ctrl - WN SF | 31 | -0.0006 | 0.1604 | ns |
| WN SF - MN SF | 31 | -0.0060 | <0.0001 | **** |

Globulin (GLOB)

| | | | | |
|-------------------|----|---------|--------|----|
| MN Ctrl - MN SF | 31 | -0.0007 | 1.0000 | ns |
| WN Ctrl - MN Ctrl | 31 | -0.0461 | 0.0096 | ** |
| WN Ctrl - WN SF | 31 | -0.0164 | 0.6860 | ns |
| WN SF - MN SF | 31 | -0.0304 | 0.0341 | * |

Glucose (GLU)

| | | | | |
|-------------------|----|---------|---------|------|
| MN Ctrl - MN SF | 31 | -1.1120 | 0.2747 | ns |
| WN Ctrl - MN Ctrl | 31 | 4.7185 | <0.0001 | **** |
| WN Ctrl - WN SF | 31 | 1.4230 | 0.1647 | ns |
| WN SF - MN SF | 31 | 3.1947 | 0.0032 | ** |

total protein (TP)

| | | | | |
|-------------------|----|---------|---------|------|
| MN Ctrl - MN SF | 31 | -0.7890 | 0.4361 | ns |
| WN Ctrl - MN Ctrl | 31 | 4.1440 | 0.0002 | *** |
| WN Ctrl - WN SF | 31 | -1.2600 | 0.2171 | ns |
| WN SF - MN SF | 31 | 5.9134 | <0.0001 | **** |

urea nitrogen (BUN)

| | | | | |
|-------------------|----|---------|---------|------|
| MN Ctrl - MN SF | 31 | 5.3366 | <0.0001 | **** |
| WN Ctrl - MN Ctrl | 31 | 6.1261 | <0.0001 | **** |
| WN Ctrl - WN SF | 31 | -0.6961 | 0.4915 | ns |
| WN SF - MN SF | 31 | 14.9929 | <0.0001 | **** |

Statistical power

Considering the small group sizes (Appendix tables 126), we wanted to ensure that the study design was not significantly underpowered. Thus, we performed a retrospective sample size and power analysis on the data by target.

Effect size estimation based on partial η^2

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 134 showed the respective effect sizes. Note that upper ends of the confidence intervals were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial η^2 values from the effect size calculation were then used for the retrospective power calculations.

Appendix Table 134
Effect size

| Parameter | Eta2_partial | CI_low | CI_high | Effect Size |
|--------------------------------|--------------|--------|---------|-------------|
| alanine aminotransferase (ALT) | | | | |
| Diet | 0.0539 | 0.0000 | 1 | small |
| Route | 0.0471 | 0.0000 | 1 | small |
| Diet:Route | 0.0771 | 0.0000 | 1 | medium |
| albumin (ALB) | | | | |
| Diet | 0.6994 | 0.5399 | 1 | large |
| Route | 0.0147 | 0.0000 | 1 | small |
| Diet:Route | 0.0000 | 0.0000 | 1 | very small |
| alkaline phosphatase (ALP) | | | | |
| Diet | 0.1379 | 0.0063 | 1 | medium |
| Route | 0.2028 | 0.0337 | 1 | large |
| Diet:Route | 0.1081 | 0.0000 | 1 | medium |
| amylase (AMY) | | | | |
| Diet | 0.8626 | 0.7822 | 1 | large |
| Route | 0.0143 | 0.0000 | 1 | small |
| Diet:Route | 0.0483 | 0.0000 | 1 | small |
| Globulin (GLOB) | | | | |
| Diet | 0.3390 | 0.1263 | 1 | large |
| Route | 0.0135 | 0.0000 | 1 | small |
| Diet:Route | 0.0135 | 0.0000 | 1 | small |
| Glucose (GLU) | | | | |
| Diet | 0.5107 | 0.2977 | 1 | large |
| Route | 0.0014 | 0.0000 | 1 | very small |
| Diet:Route | 0.0938 | 0.0000 | 1 | medium |
| total protein (TP) | | | | |

| | | | | |
|---------------------|--------|--------|---|------------|
| Diet | 0.6050 | 0.4127 | 1 | large |
| Route | 0.0632 | 0.0000 | 1 | medium |
| Diet:Route | 0.0033 | 0.0000 | 1 | very small |
| urea nitrogen (BUN) | | | | |
| Diet | 0.8634 | 0.7835 | 1 | large |
| Route | 0.2614 | 0.0680 | 1 | large |
| Diet:Route | 0.3725 | 0.1554 | 1 | large |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power in once data. For small mean differences within data with a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. In most instances, proposed sample sizes based on our data, suggested that actual sample sizes were frequently too small for a chance of detecting statistical significant differences, particularly, for the interaction of predictors (Appendix tables 135).

Appendix Table 135
Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|---|-------|----------------|-------|-----|------------|
| alanine aminotransferase (ALT) - Diet | | | | | |
| Diet | 0.8 | 140 | 7.96 | 1 | 137.61 |
| alanine aminotransferase (ALT) - Route | | | | | |
| Route | 0.8 | 161 | 7.94 | 1 | 158.61 |
| alanine aminotransferase (ALT) - Diet:Route | | | | | |
| Diet | 0.8 | 96 | 8.02 | 1 | 91.94 |
| Route | 0.8 | 96 | 8.02 | 1 | 91.94 |
| Diet:Route | 0.8 | 96 | 8.02 | 1 | 91.94 |
| albumin (ALB) - Diet | | | | | |
| Diet | 0.8 | 7 | 14.05 | 1 | 4.04 |
| albumin (ALB) - Route | | | | | |
| Route | 0.8 | 529 | 7.88 | 1 | 526.34 |
| albumin (ALB) - Diet:Route | | | | | |
| Diet | 0.8 | 1592383 | 7.85 | 1 | 1592379.00 |
| Route | 0.8 | 1592383 | 7.85 | 1 | 1592379.00 |
| Diet:Route | 0.8 | 1592383 | 7.85 | 1 | 1592379.00 |

| | | | | | |
|---|-----|-------------|-------|---|---------|
| alkaline phosphatase (ALP) - Diet | | | | | |
| Diet | 0.8 | 52 | 8.17 | 1 | 49.08 |
| alkaline phosphatase (ALP) - Route | | | | | |
| Route | 0.8 | 33 | 8.37 | 1 | 30.89 |
| alkaline phosphatase (ALP) - Diet:Route | | | | | |
| Diet | 0.8 | 67 | 8.10 | 1 | 62.77 |
| Route | 0.8 | 67 | 8.10 | 1 | 62.77 |
| Diet:Route | 0.8 | 67 | 8.10 | 1 | 62.77 |
| amylase (AMY) - Diet | | | | | |
| Diet | 0.8 | 5 | 26.55 | 1 | 2.23 |
| amylase (AMY) - Route | | | | | |
| Route | 0.8 | 543 | 7.88 | 1 | 540.71 |
| amylase (AMY) - Diet:Route | | | | | |
| Diet | 0.8 | 157 | 7.95 | 1 | 152.68 |
| Route | 0.8 | 157 | 7.95 | 1 | 152.68 |
| Diet:Route | 0.8 | 157 | 7.95 | 1 | 152.68 |
| Globulin (GLOB) - Diet | | | | | |
| Diet | 0.8 | 18 | 8.95 | 1 | 15.45 |
| Globulin (GLOB) - Route | | | | | |
| Route | 0.8 | 578 | 7.88 | 1 | 575.62 |
| Globulin (GLOB) - Diet:Route | | | | | |
| Diet | 0.8 | 578 | 7.88 | 1 | 573.62 |
| Route | 0.8 | 578 | 7.88 | 1 | 573.62 |
| Diet:Route | 0.8 | 578 | 7.88 | 1 | 573.62 |
| Glucose (GLU) - Diet | | | | | |
| Diet | 0.8 | 10 | 10.30 | 1 | 7.87 |
| Glucose (GLU) - Route | | | | | |
| Route | 0.8 | 5683 | 7.85 | 1 | 5680.50 |
| Glucose (GLU) - Diet:Route | | | | | |
| Diet | 0.8 | 78 | 8.06 | 1 | 73.89 |
| Route | 0.8 | 78 | 8.06 | 1 | 73.89 |

| | | | | | |
|----------------------------------|-----|-------------|-------|---|---------|
| Diet:Route | 0.8 | 78 | 8.06 | 1 | 73.89 |
| total protein (TP) - Diet | | | | | |
| Diet | 0.8 | 8 | 11.67 | 1 | 5.62 |
| total protein (TP) - Route | | | | | |
| Route | 0.8 | 119 | 7.98 | 1 | 116.25 |
| total protein (TP) - Diet:Route | | | | | |
| Diet | 0.8 | 2341 | 7.86 | 1 | 2336.38 |
| Route | 0.8 | 2341 | 7.86 | 1 | 2336.38 |
| Diet:Route | 0.8 | 2341 | 7.86 | 1 | 2336.38 |
| urea nitrogen (BUN) - Diet | | | | | |
| Diet | 0.8 | 5 | 26.69 | 1 | 2.22 |
| urea nitrogen (BUN) - Route | | | | | |
| Route | 0.8 | 25 | 8.59 | 1 | 22.26 |
| urea nitrogen (BUN) - Diet:Route | | | | | |
| Diet | 0.8 | 16 | 9.35 | 1 | 11.76 |
| Route | 0.8 | 16 | 9.35 | 1 | 11.76 |
| Diet:Route | 0.8 | 16 | 9.35 | 1 | 11.76 |

Retrospective calculation of statistical power in our data analysis

With the exception of urea nitrogen, we generally observed that our study was underpowered for the detection of a statistical significant difference, in particular, for the infection route and the interaction with “Diet” (Appendix Table 136). Even so, excessively large proposed sample sizes and small retrospective statistical power can also be an indicator that there is in fact no meaningful biological difference in this instance for the infection route and, thus identifying “Diet” as the main cause of difference in target levels is a robust outcome.

Appendix Table 136
Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|--|-------------|---------|------|-----|-----|
| alanine aminotransferase (ALT) - Diet | | | | | |
| Diet | 0.28 | 35 | 2.00 | 1 | 33 |
| alanine aminotransferase (ALT) - Route | | | | | |
| Route | 0.25 | 35 | 1.73 | 1 | 33 |

alanine aminotransferase (ALT) - Diet:Route

| | | | | | |
|------------|-------------|----|------|---|----|
| Diet | 0.38 | 35 | 2.92 | 1 | 31 |
| Route | 0.38 | 35 | 2.92 | 1 | 31 |
| Diet:Route | 0.38 | 35 | 2.92 | 1 | 31 |

albumin (ALB) - Diet

| | | | | | |
|------|-------------|----|-------|---|----|
| Diet | 1.00 | 35 | 81.42 | 1 | 33 |
|------|-------------|----|-------|---|----|

albumin (ALB) - Route

| | | | | | |
|-------|-------------|----|------|---|----|
| Route | 0.11 | 35 | 0.52 | 1 | 33 |
|-------|-------------|----|------|---|----|

albumin (ALB) - Diet:Route

| | | | | | |
|------------|-------------|----|------|---|----|
| Diet | 0.05 | 35 | 0.00 | 1 | 31 |
| Route | 0.05 | 35 | 0.00 | 1 | 31 |
| Diet:Route | 0.05 | 35 | 0.00 | 1 | 31 |

alkaline phosphatase (ALP) - Diet

| | | | | | |
|------|-------------|----|------|---|----|
| Diet | 0.63 | 35 | 5.60 | 1 | 33 |
|------|-------------|----|------|---|----|

alkaline phosphatase (ALP) - Route

| | | | | | |
|-------|-------------|----|------|---|----|
| Route | 0.82 | 35 | 8.90 | 1 | 33 |
|-------|-------------|----|------|---|----|

alkaline phosphatase (ALP) - Diet:Route

| | | | | | |
|------------|-------------|----|------|---|----|
| Diet | 0.51 | 35 | 4.24 | 1 | 31 |
| Route | 0.51 | 35 | 4.24 | 1 | 31 |
| Diet:Route | 0.51 | 35 | 4.24 | 1 | 31 |

amylase (AMY) - Diet

| | | | | | |
|------|-------------|----|--------|---|----|
| Diet | 1.00 | 35 | 219.66 | 1 | 33 |
|------|-------------|----|--------|---|----|

amylase (AMY) - Route

| | | | | | |
|-------|-------------|----|------|---|----|
| Route | 0.11 | 35 | 0.51 | 1 | 33 |
|-------|-------------|----|------|---|----|

amylase (AMY) - Diet:Route

| | | | | | |
|------------|-------------|----|------|---|----|
| Diet | 0.25 | 35 | 1.78 | 1 | 31 |
| Route | 0.25 | 35 | 1.78 | 1 | 31 |
| Diet:Route | 0.25 | 35 | 1.78 | 1 | 31 |

Globulin (GLOB) - Diet

| | | | | | |
|------|-------------|----|-------|---|----|
| Diet | 0.98 | 35 | 17.95 | 1 | 33 |
|------|-------------|----|-------|---|----|

Globulin (GLOB) - Route

| | | | | | |
|----------------------------------|-------------|----|--------|---|----|
| Route | 0.10 | 35 | 0.48 | 1 | 33 |
| Globulin (GLOB) - Diet:Route | | | | | |
| Diet | 0.10 | 35 | 0.48 | 1 | 31 |
| Route | 0.10 | 35 | 0.48 | 1 | 31 |
| Diet:Route | 0.10 | 35 | 0.48 | 1 | 31 |
| Glucose (GLU) - Diet | | | | | |
| Diet | 1.00 | 35 | 36.53 | 1 | 33 |
| Glucose (GLU) - Route | | | | | |
| Route | 0.06 | 35 | 0.05 | 1 | 33 |
| Glucose (GLU) - Diet:Route | | | | | |
| Diet | 0.45 | 35 | 3.62 | 1 | 31 |
| Route | 0.45 | 35 | 3.62 | 1 | 31 |
| Diet:Route | 0.45 | 35 | 3.62 | 1 | 31 |
| total protein (TP) - Diet | | | | | |
| Diet | 1.00 | 35 | 53.61 | 1 | 33 |
| total protein (TP) - Route | | | | | |
| Route | 0.32 | 35 | 2.36 | 1 | 33 |
| total protein (TP) - Diet:Route | | | | | |
| Diet | 0.06 | 35 | 0.12 | 1 | 31 |
| Route | 0.06 | 35 | 0.12 | 1 | 31 |
| Diet:Route | 0.06 | 35 | 0.12 | 1 | 31 |
| urea nitrogen (BUN) - Diet | | | | | |
| Diet | 1.00 | 35 | 221.26 | 1 | 33 |
| urea nitrogen (BUN) - Route | | | | | |
| Route | 0.93 | 35 | 12.39 | 1 | 33 |
| urea nitrogen (BUN) - Diet:Route | | | | | |
| Diet | 0.99 | 35 | 20.77 | 1 | 31 |
| Route | 0.99 | 35 | 20.77 | 1 | 31 |
| Diet:Route | 0.99 | 35 | 20.77 | 1 | 31 |

Panel c

Data analysis

Here, we present statistical comparison of systemic heme-oxygenase-1 of a total of $N=67$ well-nourished (WN) or malnourished (MN) BALB/c mice infected by sand fly bite (SF) or not (Ctrl) (Appendix table 137).

Appendix Table 137
Summary information

| Targets | Total N | WN_Ctrl | MN_Ctrl | WN_SF | MN_SF | Transformation |
|-----------|---------|---------|---------|-------|-------|---------------------------|
| Figure 4c | 67 | 15 | 8 | 21 | 23 | Box-Cox power Transformed |

We needed to analyze the data with a two-way approach to account for the two predictors, “Diet” and “Route”, both of which were between-subject factors.

For a two-way ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance
- No significant outliers

Assumption analyses

Please, note that all assumption test results shown were post data transformation, where applicable (Appendix table 137).

Data normality

The assessment of the untransformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by both predictors. Note that all groups consisted of <30 data points (range[8 to 23]) (Appendix table 137), which made groups too small to assess data distribution reliably by Shapiro-Wilks test. We executed the test anyway as an indicator of gross departure of data normality. Thus, we performed the analyses by Shapiro-Wilks test (Appendix table 138) and QQ-plots (Fig.S1a-1). We found a departure of normality for the malnourished, sand fly infected group, which was improved by data transformation but not completely remedied.

Appendix Table 138
Univariate Shapiro-Wilks test results

| Diet | Route | variable | statistic | p | Outcome |
|------|-------|----------|-----------|--------|---------|
| WN | Ctrl | Counts | 0.9176 | 0.1770 | ns |
| WN | SF | Counts | 0.9522 | 0.3741 | ns |

| | | | | | |
|----|------|--------|--------|--------|------|
| MN | Ctrl | Counts | 0.9788 | 0.9569 | ns |
| MN | SF | Counts | 0.9026 | 0.0286 | sig. |

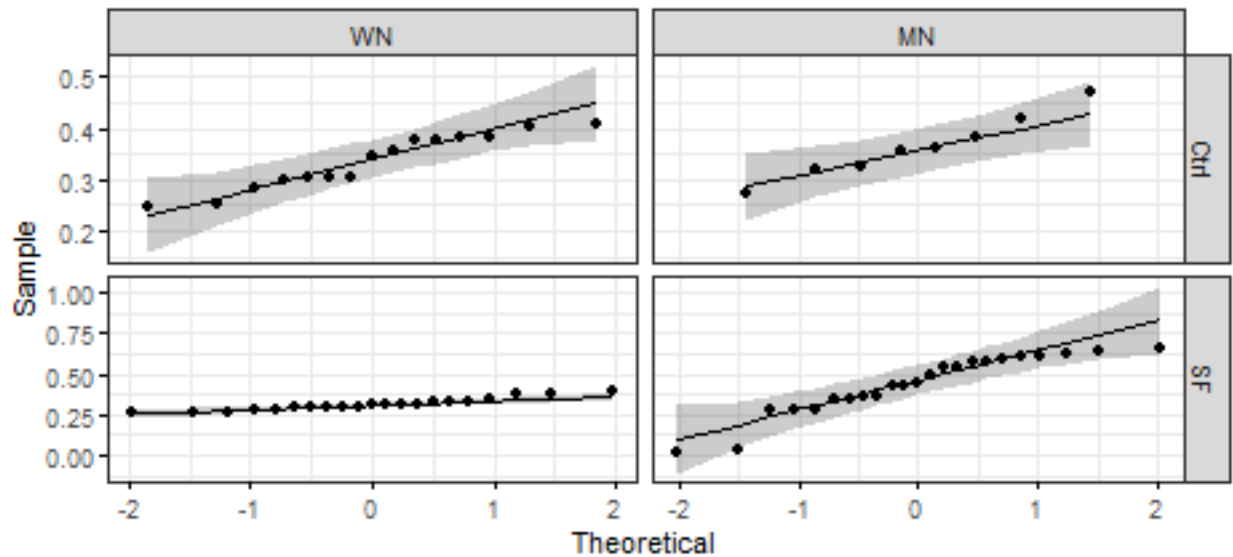


Fig.4c-1: QQ-plots of Heme-oxygenase-1 data

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset. The analysis output showed that assumption of homogeneity between groups was violated as the manlourished, sand fly infected group had much larger variance than other three groups (Appendix table 139).

Appendix Table 139

Assessment of homogeneity of variance by target

| Targets | df1 | df2 | statistic | p | sig. |
|-----------|-----|-----|-----------|---------|------|
| Figure 4c | 3 | 63 | 13.654 | <0.0001 | **** |

Outliers

A total of one outlier was observed, but not an extreme one (Appendix table 140).

Appendix Table 140

List of possible outliers

| Diet | Route | Tissue | is.outlier | is.extreme |
|------|-------|--------|------------|------------|
| WN | SF | Serum | TRUE | FALSE |

Two-way analysis

Based on the assumption analysis, we applied an appropriate two-way test to the respective datasets (Appendix table 141).

Appendix Table 141

Applied tests

| Two-way test | One-way test | Pairwise |
|----------------------|----------------------|----------------------------|
| Robust two-way ANOVA | Robust one-way ANOVA | Linear contrast expression |

The respective two-way analyses showed that statistical significance for “Diet” and its interaction with “Route”, but not for “Route” by itself (Appendix table 142), suggesting that malnourishment significantly increased HO-1 expression.

Appendix Table 142

Two-way analysis

| Effect | DFn | DFd | Statistic | p.value | p<.05 | ges |
|------------|-----|-----|-----------|---------|-------|-----|
| Diet | 1 | 31 | 12.2698 | 0.002 | ** | NA |
| Route | 1 | 31 | 2.0016 | 0.170 | | NA |
| Diet:Route | 1 | 31 | 7.0260 | 0.014 | * | NA |

For the analysis of the simple main effect for each respective between-subject factor for each target, we performed one-way analyses with the data split by always by the predictor that was not used as within the function. The results showed statistical significance when both malnourished groups were compared (Appendix table 143).

Appendix Table 143

Simple main effect analysis

| Factor | Effect | statistic | df1 | df2 | p.value | Sig. | effsize |
|--------|--------|-----------|-----|---------|---------|------|---------|
| WN | Route | 2.0470 | 1 | 19.3826 | 0.1684 | ns | 0.4332 |
| MN | Route | 5.5716 | 1 | 24.9806 | 0.0264 | * | 0.3924 |
| Ctrl | Diet | 1.0120 | 1 | 14.0782 | 0.3314 | ns | 0.3640 |
| SF | Diet | 17.8952 | 1 | 20.4862 | 0.0004 | *** | 0.7159 |

Since both predictors had only two levels each, the pairwise comparison reflected the observations of the one-way analyses above (Table 144).

Appendix Table 144
Pairwise comparison

| contrast | df | statistic | p.value | Sig. |
|-------------------|----|-----------|---------|------|
| MN Ctrl - MN SF | 31 | -0.0950 | 0.0264 | * |
| WN Ctrl - MN Ctrl | 31 | -0.0271 | 0.3314 | ns |
| WN Ctrl - WN SF | 31 | 0.0260 | 0.1684 | ns |
| WN SF - MN SF | 31 | -0.1480 | 0.0004 | *** |

Statistical power

Considering the small group sizes (Appendix tables 137), we wanted to ensure that the study design was not significantly statistically underpowered. Thus, we performed a retrospective sample size and power analysis on the data by target.

Effect size estimation based on partial eta²

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 145 shows the respective effect sizes. Note that upper ends of the confidence intervals were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial eta² values from the effect size calculation were then used for the retrospective power calculations.

Appendix Table 145
Effect size

| Parameter | Eta2_partial | CI_low | CI_high | Effect Size |
|------------|--------------|--------|---------|-------------|
| Figure 4c | | | | |
| Diet | 0.1188 | 0.0225 | 1 | medium |
| Route | 0.0548 | 0.0000 | 1 | small |
| Diet:Route | 0.0799 | 0.0063 | 1 | medium |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power in once data. For small mean differences within data of a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. In most instances, proposed sample sizes based on our data suggested that actual sample sizes were frequently too small for a chance of detecting statistical significant differences, particularly, for infection “Route” (Appendix tables 146).

Appendix Table 146
Minimum optimal sample size calculation

| Parameters | effect | power | n.total | ncp | df1 | df2 |
|------------|------------|-------|------------|------|-----|--------|
| Diet | Diet | 0.8 | 61 | 8.12 | 1 | 58.19 |
| Route | Route | 0.8 | 138 | 7.96 | 1 | 135.39 |
| Diet:Route | Diet | 0.8 | 93 | 8.02 | 1 | 88.38 |
| Diet:Route | Route | 0.8 | 93 | 8.02 | 1 | 88.38 |
| Diet:Route | Diet:Route | 0.8 | 93 | 8.02 | 1 | 88.38 |

Retrospective calculation of statistical power in our data analysis

The retrospective power calculation suggested that the study was underpowered, but not terribly (Appendix Table 147). Even so, excessively large proposed sample sizes and small retrospective statistical power can also be an indicator that there was in fact no meaningful biological difference to be found. Considering that statistical significant difference were still found, suggested that the above observations were real.

Appendix Table 147
Statistical power of data

| Parameters | effect | power | n.total | ncp | df1 | df2 |
|------------|------------|-------------|---------|------|-----|-----|
| Diet | Diet | 0.84 | 67 | 9.03 | 1 | 65 |
| Route | Route | 0.49 | 67 | 3.88 | 1 | 65 |
| Diet:Route | Diet | 0.66 | 67 | 5.82 | 1 | 63 |
| Diet:Route | Route | 0.66 | 67 | 5.82 | 1 | 63 |
| Diet:Route | Diet:Route | 0.66 | 67 | 5.82 | 1 | 63 |

Panel d

Data analysis

Here, we present statistical comparison of serum cytokine concentrations (in pg/ml) for a total of $N=38$ well-nourished (WN) or malnourished (MN) BALB/c mice infected by sand fly bite (SF) or not (Ctrl) (Appendix table 148). A total of 11 targets were measured (IFN-gamma, IL-10, IL-12p70, IL-17A, IL-18, IL-2, IL-22, IL-4, IL-5, IL-6 & TNF-alpha) per mouse.

Appendix Table 148
Summary information

| Targets | Total N | WN_Ctrl | MN_Ctrl | WN_SF | MN_SF | Transformation |
|-----------|---------|---------|---------|-------|-------|---------------------------|
| IFN-gamma | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-10 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |

| | | | | | | |
|-----------|----|---|---|----|----|---------------------------|
| IL-12p70 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-17A | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-18 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-2 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-22 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-4 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-5 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| IL-6 | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |
| TNF-alpha | 38 | 7 | 7 | 11 | 13 | Box-Cox power Transformed |

We needed to analyze the data with a two-way approach to account for the two predictors, “Diet” and “Route”, both of which were between-subject factors.

For a two-way ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance
- No significant outliers

Assumption analyses

Please, note that all assumption test results shown were post data transformation, where applicable (Appendix table 148).

Data normality

The assessment of the untransformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by both predictors. Note that all groups consisted of <30 data points (range[7 to 13]) (Appendix table 148), which made groups too small to assess data distribution reliably by Shapiro-Wilks test. We executed the test anyway as an indicator of gross departure of data normality. Thus, we performed the analyses by Shapiro-Wilks test (Appendix table 149) and QQ-plots (Fig.S1a-1). We found occasional departure of normality, which could not all be remedied by data transformation.

Appendix Table 149
Univariate Shapiro-Wilks test results

| Diet | Route | variable | statistic | p | Outcome |
|-----------|-------|----------|-----------|--------|---------|
| IFN-gamma | | | | | |
| WN | Ctrl | Counts | 0.9476 | 0.7075 | ns |
| WN | SF | Counts | 0.9163 | 0.2890 | ns |
| MN | Ctrl | Counts | 0.9088 | 0.3873 | ns |
| MN | SF | Counts | 0.9848 | 0.9951 | ns |

IL-10

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.9146 | 0.4288 | ns |
| WN | SF | Counts | 0.9234 | 0.3476 | ns |
| MN | Ctrl | Counts | 0.9419 | 0.6557 | ns |
| MN | SF | Counts | 0.8749 | 0.0608 | ns |

IL-12p70

| | | | | | |
|----|------|--------|--------|--------|------|
| WN | Ctrl | Counts | 0.8118 | 0.0535 | ns |
| WN | SF | Counts | 0.8217 | 0.0182 | sig. |
| MN | Ctrl | Counts | 0.8917 | 0.2838 | ns |
| MN | SF | Counts | 0.8981 | 0.1261 | ns |

IL-17A

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.8546 | 0.1353 | ns |
| WN | SF | Counts | 0.8886 | 0.1336 | ns |
| MN | Ctrl | Counts | 0.9320 | 0.5683 | ns |
| MN | SF | Counts | 0.9724 | 0.9212 | ns |

IL-18

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.9540 | 0.7661 | ns |
| WN | SF | Counts | 0.9226 | 0.3411 | ns |
| MN | Ctrl | Counts | 0.9828 | 0.9721 | ns |
| MN | SF | Counts | 0.8763 | 0.0636 | ns |

IL-2

| | | | | | |
|----|------|--------|--------|--------|------|
| WN | Ctrl | Counts | 0.9561 | 0.7851 | ns |
| WN | SF | Counts | 0.9565 | 0.7270 | ns |
| MN | Ctrl | Counts | 0.8783 | 0.2190 | ns |
| MN | SF | Counts | 0.8492 | 0.0278 | sig. |

IL-22

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.8472 | 0.1158 | ns |
| WN | SF | Counts | 0.9432 | 0.5584 | ns |
| MN | Ctrl | Counts | 0.8818 | 0.2347 | ns |
| MN | SF | Counts | 0.9459 | 0.5382 | ns |

IL-4

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.8578 | 0.1445 | ns |
| WN | SF | Counts | 0.9244 | 0.3567 | ns |
| MN | Ctrl | Counts | 0.9393 | 0.6326 | ns |
| MN | SF | Counts | 0.9397 | 0.4530 | ns |

IL-5

| | | | | | |
|----|------|--------|--------|--------|------|
| WN | Ctrl | Counts | 0.9194 | 0.4648 | ns |
| WN | SF | Counts | 0.9288 | 0.3986 | ns |
| MN | Ctrl | Counts | 0.9708 | 0.9042 | ns |
| MN | SF | Counts | 0.8572 | 0.0354 | sig. |

IL-6

| | | | | | |
|----|------|--------|--------|--------|------|
| WN | Ctrl | Counts | 0.8487 | 0.1195 | ns |
| WN | SF | Counts | 0.8269 | 0.0213 | sig. |
| MN | Ctrl | Counts | 0.7714 | 0.0211 | sig. |
| MN | SF | Counts | 0.9486 | 0.5777 | ns |

TNF-alpha

| | | | | | |
|----|------|--------|--------|--------|----|
| WN | Ctrl | Counts | 0.8392 | 0.0976 | ns |
| WN | SF | Counts | 0.9171 | 0.2950 | ns |
| MN | Ctrl | Counts | 0.8987 | 0.3229 | ns |
| MN | SF | Counts | 0.9372 | 0.4218 | ns |

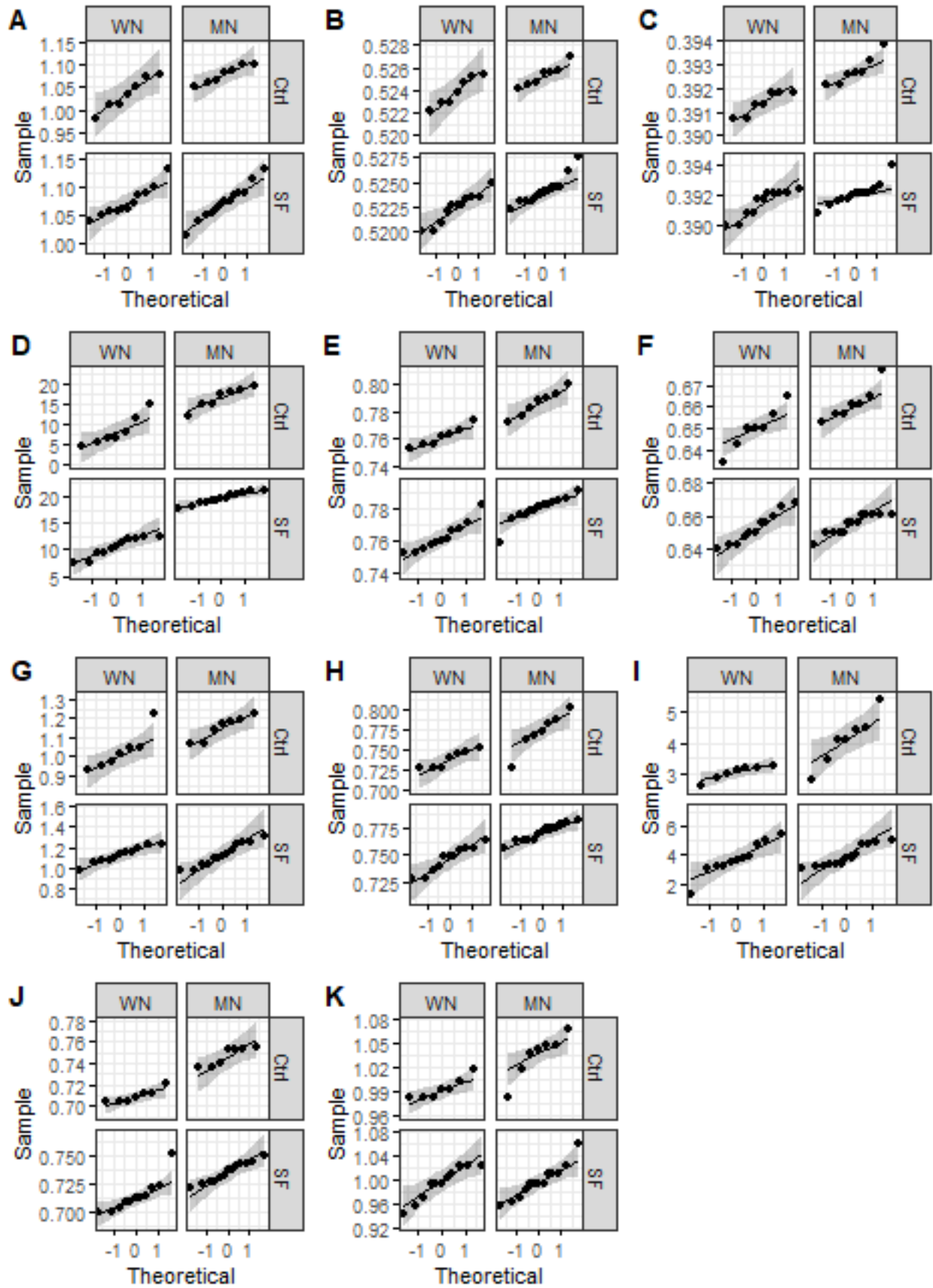


Fig.4d-1: QQ-plots of serum cytokine data: A) IFN-gamma, B) IL-10, C) IL-12p70, D) IL-17A, E) IL-18, F) IL-2, G) IL-22, H) IL-4, I) IL-5, J) IL-6 and K) TNF-alpha

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset. The analysis output showed that assumption of homogeneity between groups held for all cytokines (Appendix table 150).

Appendix Table 150
Assessment of homogeneity of variance by target

| Targets | df1 | df2 | statistic | p | sig. |
|-----------|-----|-----|-----------|--------|------|
| IFN-gamma | 3 | 34 | 0.5093 | 0.6786 | ns |
| IL-10 | 3 | 34 | 0.2930 | 0.8302 | ns |
| IL-12p70 | 3 | 34 | 0.7980 | 0.5036 | ns |
| IL-17A | 3 | 34 | 1.7416 | 0.1770 | ns |
| IL-18 | 3 | 34 | 0.3842 | 0.7650 | ns |
| IL-2 | 3 | 34 | 0.5862 | 0.6282 | ns |
| IL-22 | 3 | 34 | 0.8989 | 0.4518 | ns |
| IL-4 | 3 | 34 | 2.1753 | 0.1090 | ns |
| IL-5 | 3 | 34 | 1.9035 | 0.1476 | ns |
| IL-6 | 3 | 34 | 0.5496 | 0.6519 | ns |
| TNF-alpha | 3 | 34 | 0.6531 | 0.5865 | ns |

Outliers

Conversely, we detected outliers for 9 of the 11 cytokines (Appendix table 151). Only two of these outliers were classified as extreme (one each for IL-12p70 and IL-6).

Appendix Table 151
List of possible outliers

| Diet | Route | Tissue | is.outlier | is.extreme |
|----------|-------|----------|------------|------------|
| IL-10 | | | | |
| MN | SF | IL-10 | TRUE | FALSE |
| IL-12p70 | | | | |
| MN | Ctrl | IL-12p70 | TRUE | FALSE |
| MN | SF | IL-12p70 | TRUE | TRUE |
| MN | SF | IL-12p70 | TRUE | FALSE |
| IL-18 | | | | |
| MN | SF | IL-18 | TRUE | FALSE |

| | | | | |
|-----------|------|-----------|------|-------|
| IL-2 | | | | |
| WN | Ctrl | IL-2 | TRUE | FALSE |
| WN | Ctrl | IL-2 | TRUE | FALSE |
| MN | Ctrl | IL-2 | TRUE | FALSE |
| IL-22 | | | | |
| WN | Ctrl | IL-22 | TRUE | FALSE |
| IL-4 | | | | |
| MN | Ctrl | IL-4 | TRUE | FALSE |
| IL-5 | | | | |
| WN | SF | IL-5 | TRUE | FALSE |
| IL-6 | | | | |
| WN | SF | IL-6 | TRUE | TRUE |
| TNF-alpha | | | | |
| MN | Ctrl | TNF-alpha | TRUE | FALSE |
| MN | SF | TNF-alpha | TRUE | FALSE |

Two-way analysis

Based on the assumption analysis, we decided to applied an appropriate two-way tests to the respective datasets (Appendix table 152).

Appendix Table 152
Applied tests

| Targets | Two-way test | One-way test | Pairwise |
|-----------|------------------------|------------------------|-----------------------------------|
| IFN-gamma | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-10 | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-12p70 | Robust two-way ANOVA | Robust one-way ANOVA | Linear contrast expression |
| IL-17A | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-18 | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-2 | Robust two-way ANOVA | Robust one-way ANOVA | Linear contrast expression |
| IL-22 | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-4 | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| IL-5 | Robust two-way ANOVA | Robust one-way ANOVA | Linear contrast expression |
| IL-6 | Robust two-way ANOVA | Robust one-way ANOVA | Linear contrast expression |
| TNF-alpha | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |

The respective two-way analyses showed that there was no statistical significant differences detected for IL-22 (Appendix table 153), suggesting that this cytokine was not statistically significantly affected by either nutritional status and/or infection route. All other targets had detectable statistical significant differences mostly in relation to “Diet” with the exception of IFN- γ , where only the interaction term of “Diet” and “Route” was statistically significant. IL-10 and IL-17A also showed statistical significance for the infection route, but not for the interaction term. Conversely, IL-6 showed a statistically significant interaction between “Diet” and “Route”, while not for “Route” on its own. Finally, TNF- α showed statistical significance for both predictors as much as their interaction. This suggested that most serum cytokine levels were affected by the nutritional state of the mice, while some changed their serum concentration with respect to the infection.

Appendix Table 153

Two-way analysis

| Effect | DFn | DFd | Statistic | p.value | p<.05 | ges |
|------------|-----|-----|-----------|---------|-------|-------|
| IFN-gamma | | | | | | |
| Diet | 1 | 34 | 2.7010 | 0.1100 | | 0.074 |
| Route | 1 | 34 | 2.2870 | 0.1400 | | 0.063 |
| Diet:Route | 1 | 34 | 4.6710 | 0.0380 | * | 0.121 |
| IL-10 | | | | | | |
| Diet | 1 | 34 | 14.8460 | 0.0005 | * | 0.304 |
| Route | 1 | 34 | 8.1980 | 0.0070 | * | 0.194 |
| Diet:Route | 1 | 34 | 0.1240 | 0.7270 | | 0.004 |
| IL-12p70 | | | | | | |
| Diet | 1 | 31 | 10.8281 | 0.0040 | ** | NA |
| Route | 1 | 31 | 0.5867 | 0.4550 | | NA |
| Diet:Route | 1 | 31 | 3.1397 | 0.0950 | + | NA |
| IL-17A | | | | | | |
| Diet | 1 | 34 | 149.0550 | <0.0001 | * | 0.814 |
| Route | 1 | 34 | 13.2900 | 0.0009 | * | 0.281 |
| Diet:Route | 1 | 34 | 0.3000 | 0.5870 | | 0.009 |
| IL-18 | | | | | | |
| Diet | 1 | 34 | 52.9080 | <0.0001 | * | 0.609 |
| Route | 1 | 34 | 1.0700 | 0.3080 | | 0.030 |
| Diet:Route | 1 | 34 | 1.9850 | 0.1680 | | 0.055 |
| IL-2 | | | | | | |
| Diet | 1 | 31 | 6.8192 | 0.0170 | * | NA |

| | | | | | | |
|------------|---|----|---------|---------|-----|-------|
| Route | 1 | 31 | 0.1834 | 0.6740 | | NA |
| Diet:Route | 1 | 31 | 1.2226 | 0.2840 | | NA |
| IL-22 | | | | | | |
| Diet | 1 | 34 | 2.4590 | 0.1260 | | 0.067 |
| Route | 1 | 34 | 0.9990 | 0.3250 | | 0.029 |
| Diet:Route | 1 | 34 | 3.8250 | 0.0590 | | 0.101 |
| IL-4 | | | | | | |
| Diet | 1 | 34 | 40.7310 | <0.0001 | * | 0.545 |
| Route | 1 | 34 | 0.2550 | 0.6170 | | 0.007 |
| Diet:Route | 1 | 34 | 1.2570 | 0.2700 | | 0.036 |
| IL-5 | | | | | | |
| Diet | 1 | 31 | 5.3171 | 0.0330 | * | NA |
| Route | 1 | 31 | 0.5066 | 0.4860 | | NA |
| Diet:Route | 1 | 31 | 3.4406 | 0.0800 | + | NA |
| IL-6 | | | | | | |
| Diet | 1 | 31 | 77.5334 | 0.0010 | *** | NA |
| Route | 1 | 31 | 1.4924 | 0.2430 | | NA |
| Diet:Route | 1 | 31 | 4.6554 | 0.0490 | * | NA |
| TNF-alpha | | | | | | |
| Diet | 1 | 34 | 4.4970 | 0.0410 | * | 0.117 |
| Route | 1 | 34 | 5.0150 | 0.0320 | * | 0.129 |
| Diet:Route | 1 | 34 | 4.9680 | 0.0330 | * | 0.127 |

For the analysis of the simple main effect for each respective between-subject factor for each target, we performed one-way analyses with the data split by always by the predictor that was not used as within the function. The results showed that statistical significance for each cytokine, including IL-22, which had not shown statistical significance in the two-way analysis (Table 154). Results were quite varied between cytokines, frequently showing statistically significant differences between well-nourished uninfected and sand fly infected mice (IFN- γ , IL-10, IL-17A, IL-22 & IL-5) and well- and malnourished uninfected control groups showed statistical significant differences for all measured serum cytokines. The latter indicated that the nutritional status of the mice altered their steady state levels of measured cytokines, increasing them significantly in the malnourished group; a difference negated after parasite infection by sand fly.

Appendix Table 154
Simple main effect analysis

| Factor | Effect | statistic | df1 | df2 | p.value | Sig. | effsize |
|-----------|--------|-----------|-----|---------|---------|------|---------|
| IFN-gamma | | | | | | | |
| WN | Route | 6.7820 | 1 | 34.0000 | 0.0140 | * | 0.1660 |
| MN | Route | 0.1750 | 1 | 34.0000 | 0.6780 | ns | 0.0050 |
| Ctrl | Diet | 7.3710 | 1 | 34.0000 | 0.0100 | * | 0.1780 |
| SF | Diet | 0.0001 | 1 | 34.0000 | 0.9920 | ns | 0.0000 |
| IL-10 | | | | | | | |
| WN | Route | 5.0460 | 1 | 34.0000 | 0.0310 | * | 0.1290 |
| MN | Route | 3.2760 | 1 | 34.0000 | 0.0790 | ns | 0.0880 |
| Ctrl | Diet | 4.2600 | 1 | 34.0000 | 0.0470 | * | 0.1110 |
| SF | Diet | 10.7100 | 1 | 34.0000 | 0.0020 | * | 0.2400 |
| IL-12p70 | | | | | | | |
| WN | Route | 0.1683 | 1 | 11.8637 | 0.6890 | ns | 0.2715 |
| MN | Route | 7.4009 | 1 | 11.0197 | 0.0199 | * | 0.7033 |
| Ctrl | Diet | 23.5358 | 1 | 11.3978 | 0.0005 | *** | 1.0176 |
| SF | Diet | 1.8848 | 1 | 11.0264 | 0.1971 | ns | 0.4002 |
| IL-17A | | | | | | | |
| WN | Route | 4.5990 | 1 | 34.0000 | 0.0390 | * | 0.1190 |
| MN | Route | 8.9910 | 1 | 34.0000 | 0.0050 | * | 0.2090 |
| Ctrl | Diet | 48.8890 | 1 | 34.0000 | <0.0001 | * | 0.5900 |
| SF | Diet | 100.4660 | 1 | 34.0000 | <0.0001 | * | 0.7470 |
| IL-18 | | | | | | | |
| WN | Route | 0.0850 | 1 | 34.0000 | 0.7720 | ns | 0.0020 |
| MN | Route | 2.9700 | 1 | 34.0000 | 0.0940 | ns | 0.0800 |
| Ctrl | Diet | 30.7250 | 1 | 34.0000 | <0.0001 | * | 0.4750 |
| SF | Diet | 24.1680 | 1 | 34.0000 | <0.0001 | * | 0.4150 |
| IL-2 | | | | | | | |
| WN | Route | 0.2541 | 1 | 12.7323 | 0.6228 | ns | 0.2539 |
| MN | Route | 2.7345 | 1 | 9.6835 | 0.1302 | ns | 0.5742 |
| Ctrl | Diet | 5.9103 | 1 | 11.4433 | 0.0326 | * | 0.8855 |
| SF | Diet | 0.9822 | 1 | 12.2585 | 0.3408 | ns | 0.3648 |
| IL-22 | | | | | | | |
| WN | Route | 4.4090 | 1 | 34.0000 | 0.0430 | * | 0.1150 |
| MN | Route | 0.4150 | 1 | 34.0000 | 0.5240 | ns | 0.0120 |
| Ctrl | Diet | 6.2810 | 1 | 34.0000 | 0.0170 | * | 0.1560 |

| | | | | | | | |
|-----------|-------|---------|---|---------|---------|------|--------|
| SF | Diet | 0.0030 | 1 | 34.0000 | 0.9560 | ns | 0.0001 |
| IL-4 | | | | | | | |
| WN | Route | 1.3380 | 1 | 34.0000 | 0.2550 | ns | 0.0380 |
| MN | Route | 0.1740 | 1 | 34.0000 | 0.6790 | ns | 0.0050 |
| Ctrl | Diet | 22.7750 | 1 | 34.0000 | <0.0001 | * | 0.4010 |
| SF | Diet | 19.2140 | 1 | 34.0000 | 0.0001 | * | 0.3610 |
| IL-5 | | | | | | | |
| WN | Route | 6.5238 | 1 | 9.5085 | 0.0297 | * | 0.6051 |
| MN | Route | 0.4086 | 1 | 11.7601 | 0.5350 | ns | 0.2637 |
| Ctrl | Diet | 10.8185 | 1 | 6.9422 | 0.0135 | * | 0.9481 |
| SF | Diet | 0.0365 | 1 | 16.0327 | 0.8509 | ns | 0.2319 |
| IL-6 | | | | | | | |
| WN | Route | 0.2440 | 1 | 13.6262 | 0.6292 | ns | 0.3896 |
| MN | Route | 6.9094 | 1 | 11.8268 | 0.0223 | * | 0.6698 |
| Ctrl | Diet | 75.3053 | 1 | 10.4658 | <0.0001 | **** | 1.0480 |
| SF | Diet | 32.7090 | 1 | 15.9433 | <0.0001 | **** | 0.9228 |
| TNF-alpha | | | | | | | |
| WN | Route | 0.0020 | 1 | 34.0000 | 0.9670 | ns | 0.0000 |
| MN | Route | 9.9810 | 1 | 34.0000 | 0.0030 | * | 0.2270 |
| Ctrl | Diet | 9.3580 | 1 | 34.0000 | 0.0040 | * | 0.2160 |
| SF | Diet | 0.1070 | 1 | 34.0000 | 0.7450 | ns | 0.0030 |

Since both predictors had only two levels each, the pairwise comparison reflected the observations of the one-way analyses above (Table 155).

Appendix Table 155
Pairwise comparison

| contrast | df | statistic | p.value | Sig. |
|-------------------|----|-----------|---------|------|
| IFN-gamma | | | | |
| MN Ctrl - MN SF | 34 | 0.4188 | 0.6780 | ns |
| WN Ctrl - MN Ctrl | 34 | -2.7150 | 0.0103 | * |
| WN Ctrl - WN SF | 34 | -2.6042 | 0.0136 | * |
| WN SF - MN SF | 34 | 0.0102 | 0.9919 | ns |
| IL-10 | | | | |
| MN Ctrl - MN SF | 34 | 1.8101 | 0.0791 | ns |

| | | | | |
|-------------------|----|----------|---------|------|
| WN Ctrl - MN Ctrl | 34 | -2.0639 | 0.0467 | * |
| WN Ctrl - WN SF | 34 | 2.2463 | 0.0313 | * |
| WN SF - MN SF | 34 | -3.2726 | 0.0024 | ** |
| IL-12p70 | | | | |
| MN Ctrl - MN SF | 31 | 0.0007 | 0.0199 | * |
| WN Ctrl - MN Ctrl | 31 | -0.0014 | 0.0005 | **** |
| WN Ctrl - WN SF | 31 | -0.0001 | 0.6890 | ns |
| WN SF - MN SF | 31 | -0.0005 | 0.1971 | ns |
| IL-17A | | | | |
| MN Ctrl - MN SF | 34 | -2.9985 | 0.0050 | ** |
| WN Ctrl - MN Ctrl | 34 | -6.9920 | <0.0001 | **** |
| WN Ctrl - WN SF | 34 | -2.1445 | 0.0392 | * |
| WN SF - MN SF | 34 | -10.0233 | <0.0001 | **** |
| IL-18 | | | | |
| MN Ctrl - MN SF | 34 | 1.7233 | 0.0939 | ns |
| WN Ctrl - MN Ctrl | 34 | -5.5430 | <0.0001 | **** |
| WN Ctrl - WN SF | 34 | -0.2916 | 0.7723 | ns |
| WN SF - MN SF | 34 | -4.9161 | <0.0001 | **** |
| IL-2 | | | | |
| MN Ctrl - MN SF | 31 | 0.0056 | 0.1302 | ns |
| WN Ctrl - MN Ctrl | 31 | -0.0116 | 0.0326 | * |
| WN Ctrl - WN SF | 31 | -0.0025 | 0.6228 | ns |
| WN SF - MN SF | 31 | -0.0035 | 0.3408 | ns |
| IL-22 | | | | |
| MN Ctrl - MN SF | 34 | 0.6439 | 0.5240 | ns |
| WN Ctrl - MN Ctrl | 34 | -2.5062 | 0.0172 | * |
| WN Ctrl - WN SF | 34 | -2.0998 | 0.0432 | * |
| WN SF - MN SF | 34 | -0.0550 | 0.9565 | ns |
| IL-4 | | | | |
| MN Ctrl - MN SF | 34 | 0.4177 | 0.6788 | ns |
| WN Ctrl - MN Ctrl | 34 | -4.7723 | <0.0001 | **** |
| WN Ctrl - WN SF | 34 | -1.1569 | 0.2554 | ns |
| WN SF - MN SF | 34 | -4.3833 | 0.0001 | *** |
| IL-5 | | | | |
| MN Ctrl - MN SF | 31 | 0.2460 | 0.5350 | ns |

| | | | | |
|-------------------|----|---------|---------|------|
| WN Ctrl - MN Ctrl | 31 | -1.0729 | 0.0135 | * |
| WN Ctrl - WN SF | 31 | -0.7580 | 0.0297 | * |
| WN SF - MN SF | 31 | -0.0688 | 0.8509 | ns |
| IL-6 | | | | |
| MN Ctrl - MN SF | 31 | 0.0114 | 0.0223 | * |
| WN Ctrl - MN Ctrl | 31 | -0.0367 | <0.0001 | **** |
| WN Ctrl - WN SF | 31 | -0.0020 | 0.6292 | ns |
| WN SF - MN SF | 31 | -0.0234 | <0.0001 | **** |
| TNF-alpha | | | | |
| MN Ctrl - MN SF | 34 | 3.1593 | 0.0033 | ** |
| WN Ctrl - MN Ctrl | 34 | -3.0590 | 0.0043 | ** |
| WN Ctrl - WN SF | 34 | -0.0412 | 0.9674 | ns |
| WN SF - MN SF | 34 | -0.3273 | 0.7454 | ns |

Statistical power

Considering the small group sizes (Appendix tables 148), we wanted to ensure that the study design was not significantly statistically underpowered. Thus, we performed a retrospective sample size and power analysis on the data by target.

Effect size estimation based on partial eta²

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 156 shows the respective effect sizes. Note that upper ends of the confidence intervals were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial eta² values from the effect size calculation were then used for the retrospective power calculations.

Appendix Table 156

Effect size

| Parameter | Eta2_partial | CI_low | CI_high | Effect Size |
|------------|--------------|--------|---------|-------------|
| IFN-gamma | | | | |
| Diet | 0.0221 | 0.0000 | 1 | small |
| Route | 0.0436 | 0.0000 | 1 | small |
| Diet:Route | 0.0190 | 0.0000 | 1 | small |
| IL-10 | | | | |
| Diet | 0.1642 | 0.0202 | 1 | large |
| Route | 0.0569 | 0.0000 | 1 | small |

| | | | | |
|------------|--------|--------|---|------------|
| Diet:Route | 0.0000 | 0.0000 | 1 | very small |
| IL-12p70 | | | | |
| Diet | 0.1276 | 0.0062 | 1 | medium |
| Route | 0.0030 | 0.0000 | 1 | very small |
| Diet:Route | 0.0093 | 0.0000 | 1 | very small |
| IL-17A | | | | |
| Diet | 0.5505 | 0.3554 | 1 | large |
| Route | 0.1633 | 0.0198 | 1 | large |
| Diet:Route | 0.0588 | 0.0000 | 1 | small |
| IL-18 | | | | |
| Diet | 0.2859 | 0.0927 | 1 | large |
| Route | 0.0809 | 0.0000 | 1 | medium |
| Diet:Route | 0.0933 | 0.0000 | 1 | medium |
| IL-2 | | | | |
| Diet | 0.1391 | 0.0102 | 1 | medium |
| Route | 0.0229 | 0.0000 | 1 | small |
| Diet:Route | 0.0849 | 0.0000 | 1 | medium |
| IL-22 | | | | |
| Diet | 0.0697 | 0.0000 | 1 | medium |
| Route | 0.0256 | 0.0000 | 1 | small |
| Diet:Route | 0.0138 | 0.0000 | 1 | small |
| IL-4 | | | | |
| Diet | 0.4960 | 0.2919 | 1 | large |
| Route | 0.0076 | 0.0000 | 1 | very small |
| Diet:Route | 0.0634 | 0.0000 | 1 | medium |
| IL-5 | | | | |
| Diet | 0.0728 | 0.0000 | 1 | medium |
| Route | 0.0192 | 0.0000 | 1 | small |
| Diet:Route | 0.0814 | 0.0000 | 1 | medium |
| IL-6 | | | | |
| Diet | 0.3582 | 0.1521 | 1 | large |
| Route | 0.1521 | 0.0152 | 1 | large |
| Diet:Route | 0.2372 | 0.0592 | 1 | large |
| TNF-alpha | | | | |

| | | | | |
|------------|--------|--------|---|--------|
| Diet | 0.2127 | 0.0445 | 1 | large |
| Route | 0.1328 | 0.0080 | 1 | medium |
| Diet:Route | 0.1641 | 0.0202 | 1 | large |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power in once data. For small mean differences within data of a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. In most instances, proposed sample sizes based on our data, suggested that actual sample sizes were frequently too small for a chance of detecting statistical significant differences, particularly, for the interaction of predictors (Appendix tables 157).

Appendix Table 157
Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|------------------------|-------|---------------|------|-----|-----------|
| IFN-gamma - Diet | | | | | |
| Diet | 0.8 | 350 | 7.89 | 1 | 347.23 |
| IFN-gamma - Route | | | | | |
| Route | 0.8 | 175 | 7.94 | 1 | 172.01 |
| IFN-gamma - Diet:Route | | | | | |
| Diet | 0.8 | 407 | 7.89 | 1 | 402.23 |
| Route | 0.8 | 407 | 7.89 | 1 | 402.23 |
| Diet:Route | 0.8 | 407 | 7.89 | 1 | 402.23 |
| IL-10 - Diet | | | | | |
| Diet | 0.8 | 42 | 8.24 | 1 | 39.96 |
| IL-10 - Route | | | | | |
| Route | 0.8 | 133 | 7.97 | 1 | 130.02 |
| IL-10 - Diet:Route | | | | | |
| Diet | 0.8 | 223551 | 7.85 | 1 | 223546.80 |
| Route | 0.8 | 223551 | 7.85 | 1 | 223546.80 |
| Diet:Route | 0.8 | 223551 | 7.85 | 1 | 223546.80 |
| IL-12p70 - Diet | | | | | |
| Diet | 0.8 | 56 | 8.14 | 1 | 53.65 |
| IL-12p70 - Route | | | | | |

| | | | | | |
|-----------------------|-----|-------------|-------|---|---------|
| Route | 0.8 | 2609 | 7.86 | 1 | 2606.86 |
| IL-12p70 - Diet:Route | | | | | |
| Diet | 0.8 | 840 | 7.87 | 1 | 835.89 |
| Route | 0.8 | 840 | 7.87 | 1 | 835.89 |
| Diet:Route | 0.8 | 840 | 7.87 | 1 | 835.89 |
| IL-17A - Diet | | | | | |
| Diet | 0.8 | 9 | 10.79 | 1 | 6.81 |
| IL-17A - Route | | | | | |
| Route | 0.8 | 43 | 8.24 | 1 | 40.24 |
| IL-17A - Diet:Route | | | | | |
| Diet | 0.8 | 128 | 7.97 | 1 | 123.66 |
| Route | 0.8 | 128 | 7.97 | 1 | 123.66 |
| Diet:Route | 0.8 | 128 | 7.97 | 1 | 123.66 |
| IL-18 - Diet | | | | | |
| Diet | 0.8 | 22 | 8.69 | 1 | 19.70 |
| IL-18 - Route | | | | | |
| Route | 0.8 | 92 | 8.02 | 1 | 89.16 |
| IL-18 - Diet:Route | | | | | |
| Diet | 0.8 | 79 | 8.06 | 1 | 74.28 |
| Route | 0.8 | 79 | 8.06 | 1 | 74.28 |
| Diet:Route | 0.8 | 79 | 8.06 | 1 | 74.28 |
| IL-2 - Diet | | | | | |
| Diet | 0.8 | 51 | 8.17 | 1 | 48.58 |
| IL-2 - Route | | | | | |
| Route | 0.8 | 338 | 7.89 | 1 | 335.37 |
| IL-2 - Diet:Route | | | | | |
| Diet | 0.8 | 87 | 8.04 | 1 | 82.58 |
| Route | 0.8 | 87 | 8.04 | 1 | 82.58 |
| Diet:Route | 0.8 | 87 | 8.04 | 1 | 82.58 |
| IL-22 - Diet | | | | | |
| Diet | 0.8 | 107 | 8.00 | 1 | 104.75 |

IL-22 - Route

| | | | | | |
|-------|-----|------------|------|---|--------|
| Route | 0.8 | 301 | 7.90 | 1 | 298.94 |
|-------|-----|------------|------|---|--------|

IL-22 - Diet:Route

| | | | | | |
|------------|-----|------------|------|---|--------|
| Diet | 0.8 | 562 | 7.88 | 1 | 557.09 |
| Route | 0.8 | 562 | 7.88 | 1 | 557.09 |
| Diet:Route | 0.8 | 562 | 7.88 | 1 | 557.09 |

IL-4 - Diet

| | | | | | |
|------|-----|-----------|-------|---|------|
| Diet | 0.8 | 11 | 10.14 | 1 | 8.30 |
|------|-----|-----------|-------|---|------|

IL-4 - Route

| | | | | | |
|-------|-----|-------------|------|---|---------|
| Route | 0.8 | 1023 | 7.86 | 1 | 1020.31 |
|-------|-----|-------------|------|---|---------|

IL-4 - Diet:Route

| | | | | | |
|------------|-----|------------|------|---|--------|
| Diet | 0.8 | 118 | 7.98 | 1 | 113.91 |
| Route | 0.8 | 118 | 7.98 | 1 | 113.91 |
| Diet:Route | 0.8 | 118 | 7.98 | 1 | 113.91 |

IL-5 - Diet

| | | | | | |
|------|-----|------------|------|---|-------|
| Diet | 0.8 | 102 | 8.00 | 1 | 99.95 |
|------|-----|------------|------|---|-------|

IL-5 - Route

| | | | | | |
|-------|-----|------------|------|---|--------|
| Route | 0.8 | 403 | 7.89 | 1 | 400.15 |
|-------|-----|------------|------|---|--------|

IL-5 - Diet:Route

| | | | | | |
|------------|-----|-----------|------|---|-------|
| Diet | 0.8 | 91 | 8.03 | 1 | 86.53 |
| Route | 0.8 | 91 | 8.03 | 1 | 86.53 |
| Diet:Route | 0.8 | 91 | 8.03 | 1 | 86.53 |

IL-6 - Diet

| | | | | | |
|------|-----|-----------|------|---|-------|
| Diet | 0.8 | 17 | 9.06 | 1 | 14.23 |
|------|-----|-----------|------|---|-------|

IL-6 - Route

| | | | | | |
|-------|-----|-----------|------|---|-------|
| Route | 0.8 | 46 | 8.21 | 1 | 43.75 |
|-------|-----|-----------|------|---|-------|

IL-6 - Diet:Route

| | | | | | |
|------------|-----|-----------|------|---|-------|
| Diet | 0.8 | 28 | 8.54 | 1 | 23.48 |
| Route | 0.8 | 28 | 8.54 | 1 | 23.48 |
| Diet:Route | 0.8 | 28 | 8.54 | 1 | 23.48 |

TNF-alpha - Diet

| | | | | | |
|------------------------|-----|-----------|------|---|-------|
| Diet | 0.8 | 32 | 8.40 | 1 | 29.10 |
| TNF-alpha - Route | | | | | |
| Route | 0.8 | 54 | 8.15 | 1 | 51.22 |
| TNF-alpha - Diet:Route | | | | | |
| Diet | 0.8 | 43 | 8.26 | 1 | 38.09 |
| Route | 0.8 | 43 | 8.26 | 1 | 38.09 |
| Diet:Route | 0.8 | 43 | 8.26 | 1 | 38.09 |

Retrospective calculation of statistical power in our data analysis

With the exception of urea nitrogen, we generally observed that the our study was underpowered for the detection of a statistical significant difference, in particular, for the infection route and the interaction with “Diet” (Appendix Table 158). Even so, excessively large proposed sample sizes and small retrospective statistical power can also be an indicator that there was in fact no meaningful biological difference to be found. Considering that statistical significant difference were still found so frequently, suggested that the above observations were real.

**Appendix Table 158*
Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|------------------------|-------------|---------|------|-----|-----|
| IFN-gamma - Diet | | | | | |
| Diet | 0.15 | 38 | 0.86 | 1 | 36 |
| IFN-gamma - Route | | | | | |
| Route | 0.25 | 38 | 1.73 | 1 | 36 |
| IFN-gamma - Diet:Route | | | | | |
| Diet | 0.13 | 38 | 0.74 | 1 | 34 |
| Route | 0.13 | 38 | 0.74 | 1 | 34 |
| Diet:Route | 0.13 | 38 | 0.74 | 1 | 34 |
| IL-10 - Diet | | | | | |
| Diet | 0.76 | 38 | 7.47 | 1 | 36 |
| IL-10 - Route | | | | | |
| Route | 0.31 | 38 | 2.29 | 1 | 36 |
| IL-10 - Diet:Route | | | | | |
| Diet | 0.05 | 38 | 0.00 | 1 | 34 |
| Route | 0.05 | 38 | 0.00 | 1 | 34 |

| | | | | | |
|-----------------------|-------------|----|-------|---|----|
| Diet:Route | 0.05 | 38 | 0.00 | 1 | 34 |
| IL-12p70 - Diet | | | | | |
| Diet | 0.63 | 38 | 5.56 | 1 | 36 |
| IL-12p70 - Route | | | | | |
| Route | 0.06 | 38 | 0.11 | 1 | 36 |
| IL-12p70 - Diet:Route | | | | | |
| Diet | 0.09 | 38 | 0.36 | 1 | 34 |
| Route | 0.09 | 38 | 0.36 | 1 | 34 |
| Diet:Route | 0.09 | 38 | 0.36 | 1 | 34 |
| IL-17A - Diet | | | | | |
| Diet | 1.00 | 38 | 46.53 | 1 | 36 |
| IL-17A - Route | | | | | |
| Route | 0.76 | 38 | 7.41 | 1 | 36 |
| IL-17A - Diet:Route | | | | | |
| Diet | 0.32 | 38 | 2.37 | 1 | 34 |
| Route | 0.32 | 38 | 2.37 | 1 | 34 |
| Diet:Route | 0.32 | 38 | 2.37 | 1 | 34 |
| IL-18 - Diet | | | | | |
| Diet | 0.97 | 38 | 15.21 | 1 | 36 |
| IL-18 - Route | | | | | |
| Route | 0.43 | 38 | 3.34 | 1 | 36 |
| IL-18 - Diet:Route | | | | | |
| Diet | 0.48 | 38 | 3.91 | 1 | 34 |
| Route | 0.48 | 38 | 3.91 | 1 | 34 |
| Diet:Route | 0.48 | 38 | 3.91 | 1 | 34 |
| IL-2 - Diet | | | | | |
| Diet | 0.67 | 38 | 6.14 | 1 | 36 |
| IL-2 - Route | | | | | |
| Route | 0.15 | 38 | 0.89 | 1 | 36 |
| IL-2 - Diet:Route | | | | | |
| Diet | 0.45 | 38 | 3.53 | 1 | 34 |
| Route | 0.45 | 38 | 3.53 | 1 | 34 |

| | | | | | |
|--------------------|-------------|----|-------|---|----|
| Diet:Route | 0.45 | 38 | 3.53 | 1 | 34 |
| IL-22 - Diet | | | | | |
| Diet | 0.38 | 38 | 2.85 | 1 | 36 |
| IL-22 - Route | | | | | |
| Route | 0.16 | 38 | 1.00 | 1 | 36 |
| IL-22 - Diet:Route | | | | | |
| Diet | 0.11 | 38 | 0.53 | 1 | 34 |
| Route | 0.11 | 38 | 0.53 | 1 | 34 |
| Diet:Route | 0.11 | 38 | 0.53 | 1 | 34 |
| IL-4 - Diet | | | | | |
| Diet | 1.00 | 38 | 37.40 | 1 | 36 |
| IL-4 - Route | | | | | |
| Route | 0.08 | 38 | 0.29 | 1 | 36 |
| IL-4 - Diet:Route | | | | | |
| Diet | 0.34 | 38 | 2.57 | 1 | 34 |
| Route | 0.34 | 38 | 2.57 | 1 | 34 |
| Diet:Route | 0.34 | 38 | 2.57 | 1 | 34 |
| IL-5 - Diet | | | | | |
| Diet | 0.39 | 38 | 2.98 | 1 | 36 |
| IL-5 - Route | | | | | |
| Route | 0.13 | 38 | 0.74 | 1 | 36 |
| IL-5 - Diet:Route | | | | | |
| Diet | 0.43 | 38 | 3.37 | 1 | 34 |
| Route | 0.43 | 38 | 3.37 | 1 | 34 |
| Diet:Route | 0.43 | 38 | 3.37 | 1 | 34 |
| IL-6 - Diet | | | | | |
| Diet | 0.99 | 38 | 21.21 | 1 | 36 |
| IL-6 - Route | | | | | |
| Route | 0.72 | 38 | 6.82 | 1 | 36 |
| IL-6 - Diet:Route | | | | | |
| Diet | 0.92 | 38 | 11.82 | 1 | 34 |
| Route | 0.92 | 38 | 11.82 | 1 | 34 |

| | | | | | |
|------------------------|-------------|----|-------|---|----|
| Diet:Route | 0.92 | 38 | 11.82 | 1 | 34 |
| TNF-alpha - Diet | | | | | |
| Diet | 0.88 | 38 | 10.27 | 1 | 36 |
| TNF-alpha - Route | | | | | |
| Route | 0.65 | 38 | 5.82 | 1 | 36 |
| TNF-alpha - Diet:Route | | | | | |
| Diet | 0.76 | 38 | 7.46 | 1 | 34 |
| Route | 0.76 | 38 | 7.46 | 1 | 34 |
| Diet:Route | 0.76 | 38 | 7.46 | 1 | 34 |

Supplementary Figures

Figure S1

Panel a

Data analysis

In figure S1a, we present the longitudinal weekly observation of mouse body weight pre *Leishmania donovani* infection, to assess the impact of well-nourishing and malnourishing diets on the BALB/c mouse weight. Information of a total of $N=89$ BALB/c mice (WN=44, MN=45) over the course of 7 weeks are shown here; “Week_0” being the weight before shipment, “Week_7” being the final weight before infection.

We needed to analyze the data with a two-way approach to account for the two predictors, “Time_point” was the within-subject factor, while “Diet” was the between-subject factors in the analysis with “Weight_g” being the dependent outcome variable.

For a two-way mixed ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance
- Homogeneity of Covariance
- No significant outliers
- Assumption of sphericity

Assumption analyses

Data normality

The assessment of the untransformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by both predictors. Note that all groups consisted of $N=WN=44$, $MN=45$ individuals, which made groups large enough to assess data distribution reliably by Shapiro-Wilks test. Thus, we performed the analyses by Shapiro-Wilks test (Appendix table 159) and QQ-plots (Fig.S1a-1) and found deviations from normality only at the Week_0 time point.

Appendix Table 160
Univariate Shapiro-Wilks test results

| Weeks p.i. | variable | statistic | p | Outcome |
|------------|----------|-----------|--------|---------|
| WN | | | | |
| Week_0 | Counts | 0.8924 | 0.0006 | sig. |
| Week_2 | Counts | 0.9701 | 0.3041 | ns |
| Week_3 | Counts | 0.9667 | 0.2302 | ns |
| Week_4 | Counts | 0.9807 | 0.6618 | ns |
| Week_5 | Counts | 0.9857 | 0.8545 | ns |
| Week_6 | Counts | 0.9873 | 0.9040 | ns |
| Week_7 | Counts | 0.9852 | 0.8363 | ns |
| MN | | | | |
| Week_0 | Counts | 0.8879 | 0.0004 | sig. |
| Week_2 | Counts | 0.9628 | 0.1555 | ns |
| Week_3 | Counts | 0.9512 | 0.0565 | ns |
| Week_4 | Counts | 0.9600 | 0.1218 | ns |
| Week_5 | Counts | 0.9684 | 0.2521 | ns |
| Week_6 | Counts | 0.9503 | 0.0522 | ns |
| Week_7 | Counts | 0.9647 | 0.1847 | ns |

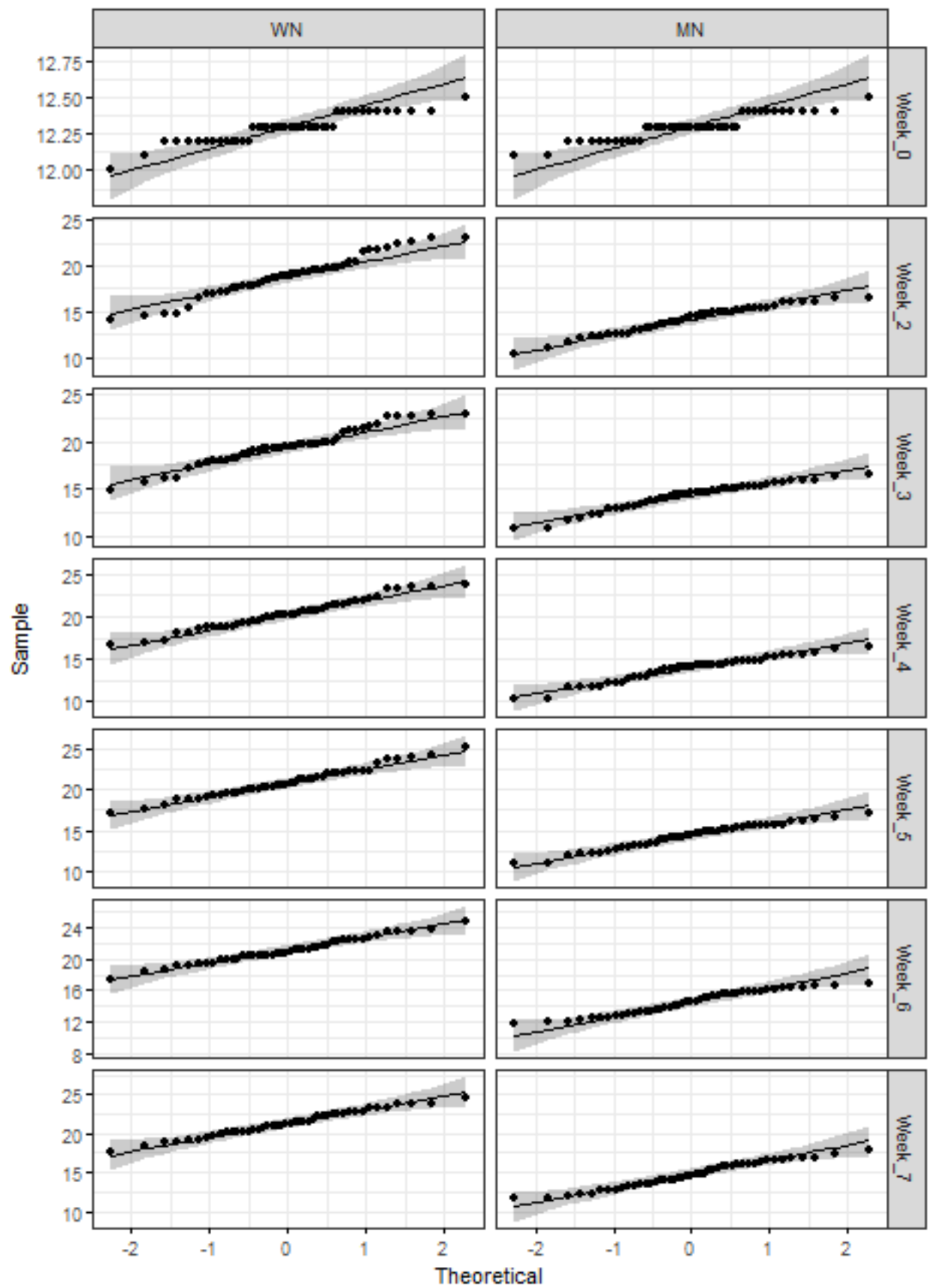


Fig.S1a-1: QQ-plots of mouse weights pre-infestation split into groups by predictor variables

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset split by the within-subject factor ("Time_point"). The analysis output showed that assumption of homogeneity between groups held for each week (Appendix table 160).

Appendix Table 161
Assessment of homogeneity of variance by week

| Weeks p.i. | df1 | df2 | statistic | p | Sig. |
|------------|-----|-----|-----------|--------|------|
| Week_0 | 1 | 87 | 0.3565 | 0.5520 | ns |
| Week_2 | 1 | 87 | 3.7582 | 0.0558 | + |
| Week_3 | 1 | 87 | 2.5150 | 0.1164 | ns |
| Week_4 | 1 | 87 | 2.0777 | 0.1531 | ns |
| Week_5 | 1 | 87 | 1.2097 | 0.2744 | ns |
| Week_6 | 1 | 87 | 0.0243 | 0.8765 | ns |
| Week_7 | 1 | 87 | 0.2668 | 0.6068 | ns |

Outliers

We found no outliers in the data (Appendix table 161).

Appendix Table 162
List of possible outliers

| |
|----------------------|
| Outcome |
| No outliers detected |

Homogeneity of covariance

There was a statistically significant violation of the assumption of homogeneity of covariance as the p-value was <0.0001. We settled for a robust two-way ANOVA to buffer some of the effect of this violation, but only low p-values can be regarded as significant (Appendix table 162).

Appendix Table 163
Box's M-test for homogeneity of covariance

| statistic | p.value | parameter |
|-----------|---------|-----------|
| 173.6597 | <0.0001 | 1 |

Sphericity

We ran a standard two-way ANOVA on the untransformed data to obtain information on the Mauchly's test of sphericity, which showed a clear violation of this assumption. This required the application of

Greenhouse-Geisser sphericity correction to the data (Appendix table 163).

Appendix Table 164
Mauchly's Test for Sphericity

| Effect | W | p | p<.05 |
|-----------------|-------|---------|-------|
| Time_point | 0.001 | <0.0001 | * |
| Diet:Time_point | 0.001 | <0.0001 | * |

Two-way mixed analysis

Based on the assumption analysis, we decided to apply a Robust two-way mixed ANOVA to the dataset to determine the effects of “Diet” and time post infection (“Time_point”) on mouse weight over time (Appendix table 164). The test output showed that both individual predictors were statistically significant, with the two-way interactions also being statistically significant.

Appendix Table 165
Robust two-way ANOVA

| Predictor | value | p.value |
|-----------------|----------|---------|
| Diet | 1473.979 | 0.001 |
| Time_point | 4255.219 | 0.001 |
| Diet:Time_point | 1474.155 | 0.001 |

For the analysis of the simple main effect for each respective between-subject factor, we performed Robust one-way ANOVA with the between-subject factor (“Diet”) split by the within-subject factor (“Time_point”). The results showed that “Diet” caused statistically significant differences with the exception of “Week_0” (Appendix table 165).

Appendix Table 166
Robust one-way ANOVA

| Weeks p.i. | test | df1 | df2 | p.value | effsize | effsize_ci_lower | effsize_ci_upper | Sig. |
|------------|----------|-----|---------|---------|---------|------------------|------------------|------|
| Week_0 | 0.0800 | 1 | 70.5479 | 0.7781 | 0.1079 | 0.0000 | 0.3362 | ns |
| Week_2 | 117.3500 | 1 | 59.3467 | <0.0001 | 1.0245 | 0.9068 | 1.1081 | **** |
| Week_3 | 196.3361 | 1 | 62.4439 | <0.0001 | 1.0807 | 1.0095 | 1.1399 | **** |
| Week_4 | 305.9447 | 1 | 65.4906 | <0.0001 | 1.1164 | 1.0687 | 1.1530 | **** |
| Week_5 | 310.8601 | 1 | 68.4596 | <0.0001 | 1.1196 | 1.0742 | 1.1517 | **** |
| Week_6 | 337.1300 | 1 | 70.9299 | <0.0001 | 1.1099 | 1.0692 | 1.1457 | **** |
| Week_7 | 287.8534 | 1 | 70.8023 | <0.0001 | 1.1042 | 1.0507 | 1.1431 | **** |

For the pairwise comparison, we applied a Linear contrast expression (Appendix table 166). Since the “Diet” predictor only had two factor levels, the output showed the same result as the Robust one-way ANOVA above (Appendix table 165).

Appendix Table 167
Robust one-way ANOVA - sig. summary

| Weeks p.i. | psihat | ci.lower | ci.upper | p.value | Sig. |
|------------|---------|----------|----------|---------|------|
| Week_0 | -0.0056 | -0.0447 | 0.0336 | 0.7781 | ns |
| Week_2 | 4.7384 | 3.8632 | 5.6135 | <0.0001 | **** |
| Week_3 | 5.2222 | 4.4773 | 5.9671 | <0.0001 | **** |
| Week_4 | 6.3616 | 5.6353 | 7.0878 | <0.0001 | **** |
| Week_5 | 6.4700 | 5.7378 | 7.2021 | <0.0001 | **** |
| Week_6 | 6.4950 | 5.7896 | 7.2003 | <0.0001 | **** |
| Week_7 | 6.6050 | 5.8287 | 7.3813 | <0.0001 | **** |

Conclusion

In conclusion, “Diet” was a potent predictor for mouse weight gain over time pre-infestation.

Panel c

We analyzed a total of $N=86$ BALB/c mice (MN=43, WN=43). These were the same mice as analyzed in figure 2a for parasite dissemination events. Here, we quantified sand fly feeding success by counting the number of visibly fed sand flies. For the data analysis we tested several Poisson and negative binomial-type regression models. Based on the Akaike information criterion (AIC) we selected a standard poisson regression model for the data analysis post data re-scaling. The model fitted the data well producing no statistically significant departure from 1 for its dispersion ratio (chisq_statistic: 82.9122, dispersion_ratio: 0.987, residual_df: 84, p_value: 0.5131), but showing only a small pseudo- R^2 (Nagelkerke (Cragg and Uhler): 0.030404). The model output showed that “Diet” was not a statistically significant predictor, suggesting that the nutritional state of the mice did not impact the sand flies ability to feed on them (Appendix table 167).

Appendix Table 168
Negative binomial regression model output

| Predictors | Estimate | Std. Error | z value | Pr(> z) | sig. |
|-------------|----------|------------|---------|----------|------|
| (Intercept) | 2.3851 | 0.0463 | 51.5431 | <0.0001 | **** |
| DietWN_SF | 0.1036 | 0.0638 | 1.6243 | 0.1043 | ns |

The pairwise comparison based on the estimated marginal means showed that sand flies fed just as well on well-nourished animals as on malnourished animals excluding any unintended parasite inoculation bias

due to the nutritional state (Appendix table 168 and 169).

Appendix Table 169
Pairwise comparison based on estimated marginal means

| Predictor pairs | estimate | SE | df | z.ratio | p.value | sig. |
|-----------------|----------|--------|-----|---------|---------|------|
| MN_SF - WN_SF | -0.1036 | 0.0638 | Inf | -1.6243 | 0.1043 | ns |

Appendix Table 170
Pairwise comparison letter code

| Diet | emmean | SE | df | asympt.LCL | asympt.UCL | .group |
|-------|--------|--------|-----|------------|------------|--------|
| MN_SF | 2.3851 | 0.0463 | Inf | 2.2814 | 2.4888 | a |
| WN_SF | 2.4888 | 0.0439 | Inf | 2.3903 | 2.5873 | a |

Figure S3

Panel c

Data analysis

Here, we present the statistical comparisons of spleen and liver weights (g), respectively, of a total of $N=77$ well-nourished (WN) or malnourished (MN) BALB/c mice, uninfected (Ctrl), infected by sand fly bite (SF) or by “needle” inoculation (Appendix table 170).

Appendix Table 171
Summary information

| Targets | Total N | WN_Ctrl | MN_Ctrl | WN_Needle | MN_Needle | WN_SF | MN_SF | Transformation |
|---------|---------|---------|---------|-----------|-----------|-------|-------|----------------|
| Liver | 77 | 15 | 10 | 10 | 11 | 15 | 16 | untransformed |
| Spleen | 77 | 15 | 10 | 10 | 11 | 15 | 16 | untransformed |

We needed to analyze the data with a two-way approach to account for the two predictors, “Diet” and “Route”, both of which were between-subject factors.

For a two-way ANOVA, we had to assess the data for compliance with assumptions:

- Data normality
- Homogeneity of variance
- No significant outliers

Assumption analyses

Please, note that all assumption test results shown are post data transformation, where applicable (Appendix table 170).

Data normality

The assessment of the untransformed data distribution for each group was conducted by Shapiro-Wilks test and QQ-plot after splitting the data by both predictors. Note that all groups consisted of <30 data points (range[10 to 16]) (Appendix table 170), which made groups too small to assess data distribution reliably by Shapiro-Wilks test. We executed the test anyway as an indicator of gross departure of data normality. Thus, we performed the analyses by Shapiro-Wilks test (Appendix table 171) and QQ-plots (Fig.S3c-1). We found no departure of normality for the liver weights, but encountered minor deviation for the well-nourished, sand fly infected and malnourished control groups for the spleen weights, respectively. However, the QQ-plot suggested that all data points fell within the 95% confidence intervals, with the exception of the well-nourished, needle inoculated group's liver weights. Any attempt by data transformation resulted in more deviation from normality in the other groups, thus, we omitted any data transformation.

Appendix Table 172
Univariate Shapiro-Wilks test results

| Diet | Route | variable | statistic | p | Outcome |
|--------|--------|----------|-----------|--------|---------|
| Liver | | | | | |
| WN | Ctrl | Counts | 0.9589 | 0.6738 | ns |
| WN | Needle | Counts | 0.9128 | 0.3009 | ns |
| WN | SF | Counts | 0.9830 | 0.9861 | ns |
| MN | Ctrl | Counts | 0.9636 | 0.8261 | ns |
| MN | Needle | Counts | 0.9299 | 0.4102 | ns |
| MN | SF | Counts | 0.9789 | 0.9540 | ns |
| Spleen | | | | | |
| WN | Ctrl | Counts | 0.9389 | 0.3685 | ns |
| WN | Needle | Counts | 0.9168 | 0.3310 | ns |
| WN | SF | Counts | 0.8736 | 0.0381 | sig. |
| MN | Ctrl | Counts | 0.8065 | 0.0174 | sig. |
| MN | Needle | Counts | 0.8682 | 0.0735 | ns |
| MN | SF | Counts | 0.9107 | 0.1195 | ns |

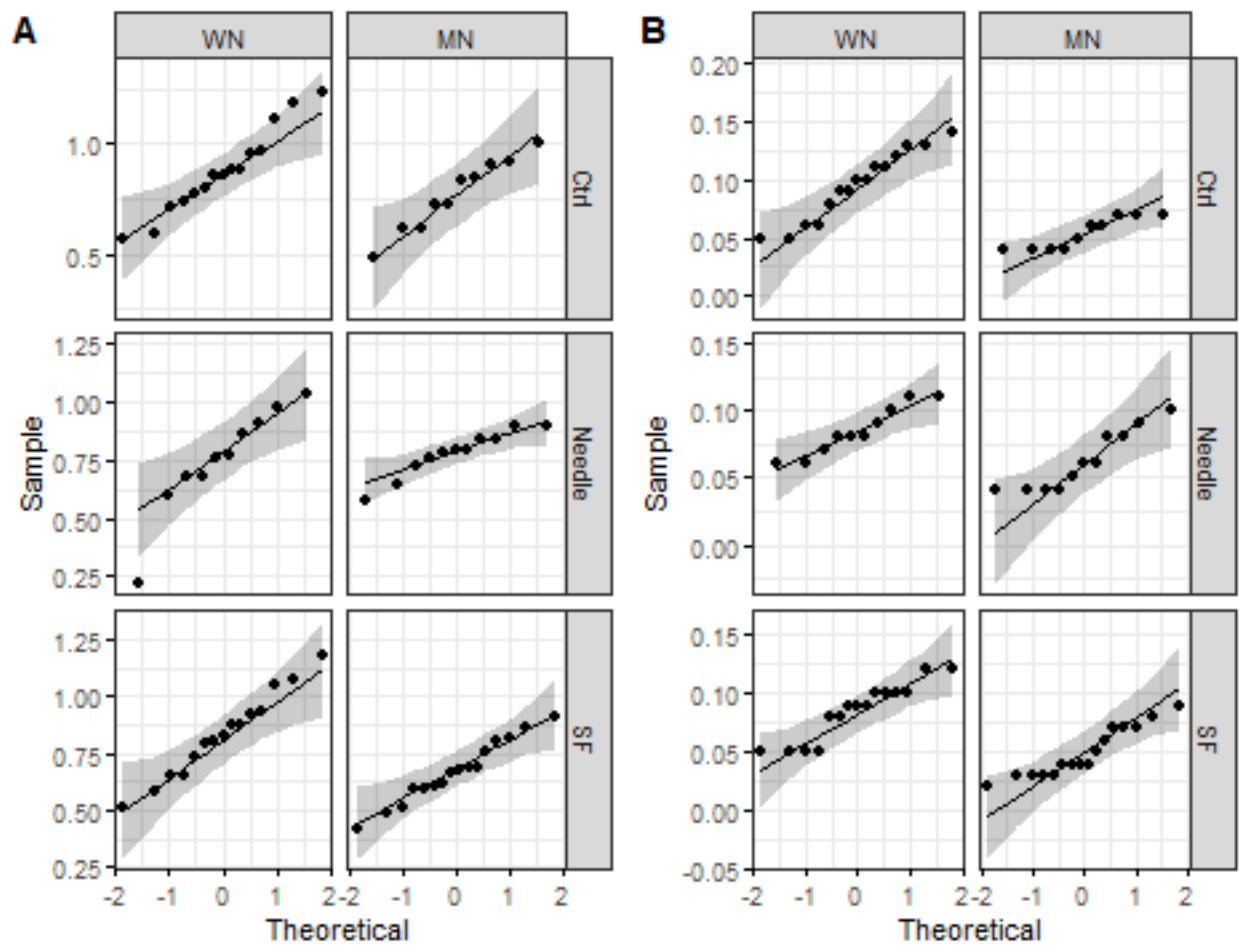


Fig.S3c-1: QQ-plots of serum chemistry targets' data: A) Liver and B) Spleen

Homogeneity of variance

The assessment of homogeneity of variance was conducted by Levene's test for the dataset. The analysis output showed that assumption of homogeneity between groups held for all organ weights (Appendix table 172).

Appendix Table 173
Assessment of homogeneity of variance by target

| Targets | df1 | df2 | statistic | p | sig. |
|---------|-----|-----|-----------|--------|------|
| Liver | 5 | 71 | 1.1097 | 0.3631 | ns |
| Spleen | 5 | 71 | 1.0924 | 0.3722 | ns |

Outliers

Conversely, we detected two outliers for the liver (Appendix table 173), non of which were extreme.

Appendix Table 174
List of possible outliers

| Diet | Route | Tissue | is.outlier | is.extreme |
|-------|--------|--------|------------|------------|
| Liver | | | | |
| WN | Needle | Liver | TRUE | FALSE |
| MN | Needle | Liver | TRUE | FALSE |

Two-way analysis

Based on the assumption analysis, we applied an appropriate two-way test to the respective datasets (Appendix table 174).

Appendix Table 175
Applied tests

| Targets | Two-way test | One-way test | Pairwise |
|---------|--------------------------|--------------------------------------|-----------------------------------|
| Liver | Standard two-way ANOVA | Standard one-way ANOVA | Estimated marginal means analysis |
| Spleen | Simple linear regression | Not applicable for linear regression | Estimated marginal means analysis |

The respective two-way analyses showed that there was only statistical significant differences with respect to the animals nutritional state (Appendix table 175), while the infection route did not make any difference with respect to the organs' weight. There was also no statistically significant interaction between both predictors.

Appendix Table 176
Two-way analysis

| Effect | DFn | DFd | Statistic | p.value | p<.05 | ges |
|------------|-----|-----|-----------|---------|-------|-------|
| Liver | | | | | | |
| Diet | 1 | 71 | 5.4720 | 0.0220 | * | 0.072 |
| Route | 2 | 71 | 1.2640 | 0.2890 | | 0.034 |
| Diet:Route | 2 | 71 | 1.8740 | 0.1610 | | 0.050 |
| Spleen | | | | | | |
| Diet | 1 | 71 | 42.3545 | <0.0001 | **** | NA |
| Route | 2 | 71 | 0.9552 | 0.3896 | | NA |
| Diet:Route | 2 | 71 | 0.9608 | 0.3875 | | NA |

The analysis of the simple main effect was only performed for the liver weights, as the spleen had been analyzed by linear regression. Each respective between-subject factor was used to perform a one-way

analyses with the data split always by the predictor that was not used as within the function. The results showed that statistical significance was only observed for the comparison of well-nourished and malnourished, sand fly infected mice (Table 176).

Appendix Table 177
Simple main effect analysis

| Factor | Effect | statistic | df1 | df2 | p.value | Sig. | effsize |
|--------|--------|-----------|-----|-----|---------|------|---------|
| Liver | | | | | | | |
| WN | Route | 1.578 | 2 | 71 | 0.214 | ns | 0.043 |
| MN | Route | 1.560 | 2 | 71 | 0.217 | ns | 0.042 |
| Ctrl | Diet | 2.373 | 1 | 71 | 0.128 | ns | 0.032 |
| Needle | Diet | 0.121 | 1 | 71 | 0.728 | ns | 0.002 |
| SF | Diet | 6.725 | 1 | 71 | 0.012 | * | 0.087 |

The pairwise comparison confirmed this observation for the liver weights showing only statistical significance for the well-nourished and malnourished, sand fly infected mice (Table 177). This suggested that only infection by sand fly altered the observed average liver weights, resulting in lower average weights in the malnourished group compared to their well-nourished counterpart. This observation was also true for the spleen weights (Table 177). However, here the uninfected control groups also showed statistical significant difference in weights.

Appendix Table 178
Pairwise comparison

| contrast | df | statistic | p.value | Sig. |
|-----------------------|----|-----------|---------|------|
| Liver | | | | |
| MN Ctrl - MN Needle | 71 | -0.1316 | 0.8957 | ns |
| MN Ctrl - MN SF | 71 | 1.3755 | 0.2600 | ns |
| MN Needle - MN SF | 71 | 1.5625 | 0.2600 | ns |
| WN Ctrl - MN Ctrl | 71 | 1.5403 | 0.1279 | ns |
| WN Ctrl - WN Needle | 71 | 1.7725 | 0.2418 | ns |
| WN Ctrl - WN SF | 71 | 0.6882 | 0.4936 | ns |
| WN Needle - MN Needle | 71 | -0.3485 | 0.7285 | ns |
| WN Needle - WN SF | 71 | -1.1570 | 0.3767 | ns |
| WN SF - MN SF | 71 | 2.5933 | 0.0115 | * |
| Spleen | | | | |
| MN Ctrl - MN Needle | 71 | -0.0078 | 0.9998 | ns |
| MN Ctrl - MN SF | 71 | 0.0046 | 1.0000 | ns |

| | | | | |
|-----------------------|----|---------|--------|-----|
| MN Ctrl - WN Needle | 71 | -0.0300 | 0.0652 | + |
| MN Needle - MN SF | 71 | 0.0124 | 0.9376 | ns |
| MN Needle - WN SF | 71 | -0.0228 | 0.1911 | ns |
| WN Ctrl - MN Ctrl | 71 | 0.0407 | 0.0007 | *** |
| WN Ctrl - MN Needle | 71 | 0.0328 | 0.0082 | ** |
| WN Ctrl - WN Needle | 71 | 0.0107 | 0.9883 | ns |
| WN Ctrl - WN SF | 71 | 0.0100 | 0.9819 | ns |
| WN Needle - MN Needle | 71 | 0.0222 | 0.3620 | ns |
| WN Needle - MN SF | 71 | 0.0346 | 0.0052 | ** |
| WN Needle - WN SF | 71 | -0.0007 | 1.0000 | ns |
| WN SF - MN SF | 71 | 0.0353 | 0.0008 | *** |

Statistical power

Considering the small group sizes (Appendix tables 170), we wanted to ensure that the study design was not significantly statistically underpowered. Thus, we performed a retrospective sample size and power analysis on the data by target.

Effect size estimation based on partial η^2

Effect sizes were calculated by predictor and the different potential interaction combinations of them. Appendix tables 178 shows the respective effect sizes. Note that upper ends of the confidence intervals were automatically set to 1 for this type of calculation. Large effect sizes reflected statistically meaningful differences in the data analysis. The partial η^2 values from the effect size calculation were then used for the retrospective power calculations.

Appendix Table 179
Effect size

| Parameter | Eta2_partial | CI_low | CI_high | Effect Size |
|------------|--------------|--------|---------|-------------|
| Liver | | | | |
| Diet | 0.0543 | 0.0004 | 1 | small |
| Route | 0.0322 | 0.0000 | 1 | small |
| Diet:Route | 0.0501 | 0.0000 | 1 | small |
| Spleen | | | | |
| Diet | 0.3479 | 0.2055 | 1 | large |
| Route | 0.0220 | 0.0000 | 1 | small |
| Diet:Route | 0.0264 | 0.0000 | 1 | small |

Retrospective minimum total sample size estimation for 80% power

The accepted rule of thumb is to have at least 80% (0.8 as ratio) statistical power in once data. For small mean differences within data of a high level of complexity, this is often hard to achieve, because of cost and ability to manage large sample sizes. In most instances, proposed sample sizes based on our data, suggested that actual sample sizes were frequently too small for a chance of detecting statistical significant differences, particularly, for the interaction of predictors (Appendix tables 179).

Appendix Table 180
Minimum optimal sample size calculation

| Effect | power | n.total | ncp | df1 | df2 |
|---------------------|-------|------------|------|-----|--------|
| Liver - Diet | | | | | |
| Diet | 0.8 | 139 | 7.96 | 1 | 136.66 |
| Liver - Route | | | | | |
| Route | 0.8 | 293 | 9.73 | 2 | 289.22 |
| Liver - Diet:Route | | | | | |
| Diet | 0.8 | 151 | 7.95 | 1 | 144.71 |
| Route | 0.8 | 186 | 9.80 | 2 | 179.62 |
| Diet:Route | 0.8 | 186 | 9.80 | 2 | 179.62 |
| Spleen - Diet | | | | | |
| Diet | 0.8 | 17 | 9.00 | 1 | 14.87 |
| Spleen - Route | | | | | |
| Route | 0.8 | 432 | 9.70 | 2 | 428.82 |
| Spleen - Diet:Route | | | | | |
| Diet | 0.8 | 292 | 7.90 | 1 | 285.95 |
| Route | 0.8 | 360 | 9.72 | 2 | 353.01 |
| Diet:Route | 0.8 | 360 | 9.72 | 2 | 353.01 |

Retrospective calculation of statistical power in our data analysis

We generally observed that our study was underpowered for the detection of a statistical significant difference, in particular, for the infection route and the interaction with “Diet” (Appendix Table 180). Even so, excessively large proposed sample sizes and small retrospective statistical power can also be an indicator that there is in fact no meaningful biological difference to be found. Considering that statistical significant difference were still found, suggested that the above observations were real.

Appendix Table 181
Statistical power of data

| Effect | power | n.total | ncp | df1 | df2 |
|---------------------|-------------|---------|-------|-----|-----|
| Liver - Diet | | | | | |
| Diet | 0.55 | 77 | 4.42 | 1 | 75 |
| Liver - Route | | | | | |
| Route | 0.27 | 77 | 2.56 | 2 | 74 |
| Liver - Diet:Route | | | | | |
| Diet | 0.51 | 77 | 4.06 | 1 | 71 |
| Route | 0.41 | 77 | 4.06 | 2 | 71 |
| Diet:Route | 0.41 | 77 | 4.06 | 2 | 71 |
| Spleen - Diet | | | | | |
| Diet | 1.00 | 77 | 41.09 | 1 | 75 |
| Spleen - Route | | | | | |
| Route | 0.19 | 77 | 1.73 | 2 | 74 |
| Spleen - Diet:Route | | | | | |
| Diet | 0.30 | 77 | 2.08 | 1 | 71 |
| Route | 0.22 | 77 | 2.08 | 2 | 71 |
| Diet:Route | 0.22 | 77 | 2.08 | 2 | 71 |

References

- [1] J. J. Allaire et al. *rmarkdown: Dynamic Documents for R*. 2024. URL: <https://github.com/rstudio/rmarkdown>.
- [2] JJ Allaire et al. *rmarkdown: Dynamic Documents for R*. R package version 2.27. 2024. URL: <https://github.com/rstudio/rmarkdown>.
- [3] Tomas J. Aragon. *epitools: Epidemiology Tools*. R package version 0.5-10.1. 2020. URL: <https://CRAN.R-project.org/package=epitools>.
- [4] Mattan S. Ben-Shachar, Daniel Lüdtke, and Dominique Makowski. “effectsize: Estimation of Effect Size Indices and Standardized Parameters”. In: *Journal of Open Source Software* 5.56 (2020), p. 2815. DOI: 10.21105/joss.02815³. URL: <https://doi.org/10.21105/joss.02815>.
- [5] Metin Bulus. *pwrss: Statistical Power and Sample Size Calculation Tools*. R package version 0.3.1. 2023. URL: <https://CRAN.R-project.org/package=pwrss>.

³<https://doi.org/10.21105/joss.02815>

- [6] Francisco Cribari-Neto and Achim Zeileis. “Beta Regression in R”. In: *Journal of Statistical Software* 34.2 (2010), pp. 1–24. doi: 10.18637/jss.v034.i02⁴.
- [7] Sam Firke. *janitor: Simple Tools for Examining and Cleaning Dirty Data*. R package version 2.2.0. 2023. URL: <https://CRAN.R-project.org/package=janitor>.
- [8] John Fox and Sanford Weisberg. *An R Companion to Applied Regression*. Third. Thousand Oaks CA: Sage, 2019. URL: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- [9] Marek Gagolewski. “stringi: Fast and portable character string processing in R”. In: *Journal of Statistical Software* 103.2 (2022), pp. 1–59. doi: 10.18637/jss.v103.i02⁵.
- [10] Philippe Grosjean and Frederic Ibanez. *pastecs: Package for Analysis of Space-Time Ecological Series*. R package version 1.4.2. 2024. URL: <https://CRAN.R-project.org/package=pastecs>.
- [11] Bettina Grün, Ioannis Kosmidis, and Achim Zeileis. “Extended Beta Regression in R: Shaken, Stirred, Mixed, and Partitioned”. In: *Journal of Statistical Software* 48.11 (2012), pp. 1–25. doi: 10.18637/jss.v048.i11⁶.
- [12] Frank E Harrell Jr. *Hmisc: Harrell Miscellaneous*. R package version 5.1-3. 2024. URL: <https://CRAN.R-project.org/package=Hmisc>.
- [13] Torsten Hothorn, Frank Bretz, and Peter Westfall. “Simultaneous Inference in General Parametric Models”. In: *Biometrical Journal* 50.3 (2008), pp. 346–363.
- [14] Alboukadel Kassambara. *ggpubr: ‘ggplot2’ Based Publication Ready Plots*. R package version 0.6.0. 2023. URL: <https://CRAN.R-project.org/package=ggpubr>.
- [15] Alboukadel Kassambara. *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.7.2. 2023. URL: <https://CRAN.R-project.org/package=rstatix>.
- [16] Lukasz Komsta and Frederick Novomestky. *moments: Moments, Cumulants, Skewness, Kurtosis and Related Tests*. R package version 0.14.1. 2022. URL: <https://CRAN.R-project.org/package=moments>.
- [17] Ioannis Kosmidis and Achim Zeileis. *Extended-Support Beta Regression for [0, 1] Responses*. Unpublished Manuscript. 2024.
- [18] Kuhn and Max. “Building Predictive Models in R Using the caret Package”. In: *Journal of Statistical Software* 28.5 (2008), pp. 1–26. doi: 10.18637/jss.v028.i05⁷. URL: <https://www.jstatsoft.org/index.php/jss/article/view/v028i05>.
- [19] Russell V. Lenth. *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version 1.10.3. 2024. URL: <https://CRAN.R-project.org/package=emmeans>.
- [20] Lesnoff et al. *aod: Analysis of Overdispersed Data*. R package version 1.3.3. 2012. URL: <https://cran.r-project.org/package=aod>.
- [21] Daniel Lüdtke. “sjmisc: Data and Variable Transformation Functions.” In: *Journal of Open Source Software* 3.26 (2018), p. 754. doi: 10.21105/joss.00754⁸.

⁴<https://doi.org/10.18637/jss.v034.i02>

⁵<https://doi.org/10.18637/jss.v103.i02>

⁶<https://doi.org/10.18637/jss.v048.i11>

⁷<https://doi.org/10.18637/jss.v028.i05>

⁸<https://doi.org/10.21105/joss.00754>

- [22] Daniel Lüdtke et al. “performance: An R Package for Assessment, Comparison and Testing of Statistical Models”. In: *Journal of Open Source Software* 6.60 (2021), p. 3139. doi: 10.21105/joss.03139⁹.
- [23] Patrick Mair and Rand Wilcox. “Robust Statistical Methods in R Using the WRS2 Package”. In: *Behavior Research Methods* 52 (2020). doi: 10.3758/s13428-019-01246-w¹⁰.
- [24] Salvatore S. Mangiafico. *rcompanion: Functions to Support Extension Education Program Evaluation*. version 2.4.36. Rutgers Cooperative Extension. New Brunswick, New Jersey, 2024. URL: <https://CRAN.R-project.org/package=rcompanion/>.
- [25] Tiago Olivoto and Alessandro Dal’Col Lúcio. “metan: An R package for multi-environment trial analysis”. In: *Methods in Ecology and Evolution* 11.6 (2020), pp. 783–789. doi: 10.1111/2041-210X.13384¹¹.
- [26] José Pinheiro, Douglas Bates, and R Core Team. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-165. 2024. URL: <https://CRAN.R-project.org/package=nlme>.
- [27] José C. Pinheiro and Douglas M. Bates. *Mixed-Effects Models in S and S-PLUS*. New York: Springer, 2000. doi: 10.1007/b98882¹².
- [28] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2024. URL: <https://www.R-project.org/>.
- [29] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2024. URL: <https://www.R-project.org/>.
- [30] Xavier Robin et al. “pROC: an open-source package for R and S+ to analyze and compare ROC curves”. In: *BMC Bioinformatics* 12 (2011), p. 77.
- [31] Francisco Rodriguez-Sanchez and Connor P. Jackson. *grateful: Facilitate citation of R packages*. 2023. URL: <https://pakillo.github.io/grateful/>.
- [32] RStudio Team. *RStudio: Integrated Development for R*. Type: Computer Program. RStudio, PBC., 2023. URL: <http://www.rstudio.com/>.
- [33] Simon Urbanek and Kent Johnson. *tiff: Read and Write TIFF Images*. R package version 0.1-12. 2023. URL: <https://CRAN.R-project.org/package=tiff>.
- [34] W. N. Venables and B. D. Ripley. *Modern Applied Statistics with S*. Fourth. ISBN 0-387-95457-0. New York: Springer, 2002. URL: <https://www.stats.ox.ac.uk/pub/MASS4/>.
- [35] Hadley Wickham. *conflicted: An Alternative Conflict Resolution Strategy*. R package version 1.2.0. 2023. URL: <https://CRAN.R-project.org/package=conflicted>.
- [36] Hadley Wickham et al. “Welcome to the tidyverse”. In: *Journal of Open Source Software* 4.43 (2019), p. 1686. doi: 10.21105/joss.01686¹³.
- [37] Yihui Xie. *bookdown: Authoring Books and Technical Documents with R Markdown*. Boca Raton, Florida: Chapman and Hall/CRC, 2016. ISBN: 978-1138700109. URL: <https://bookdown.org/yihui/bookdown>.

⁹<https://doi.org/10.21105/joss.03139>

¹⁰<https://doi.org/10.3758/s13428-019-01246-w>

¹¹<https://doi.org/10.1111/2041-210X.13384>

¹²<https://doi.org/10.1007/b98882>

¹³<https://doi.org/10.21105/joss.01686>

- [38] Yihui Xie. *bookdown: Authoring Books and Technical Documents with R Markdown*. R package version 0.40. 2024. URL: <https://github.com/rstudio/bookdown>.
- [39] Yihui Xie. *Dynamic Documents with R and knitr*. 2nd. ISBN 978-1498716963. Boca Raton, Florida: Chapman and Hall/CRC, 2015. URL: <https://yihui.org/knitr/>.
- [40] Yihui Xie. “knitr: A Comprehensive Tool for Reproducible Research in R”. In: *Implementing Reproducible Computational Research*. Ed. by Victoria Stodden, Friedrich Leisch, and Roger D. Peng. ISBN 978-1466561595. Chapman and Hall/CRC, 2014.
- [41] Yihui Xie. *knitr: A General-Purpose Package for Dynamic Report Generation in R*. R package version 1.48. 2024. URL: <https://yihui.org/knitr/>.
- [42] Yihui Xie, J.J. Allaire, and Garrett Grolemund. *R Markdown: The Definitive Guide*. Boca Raton, Florida: Chapman and Hall/CRC, 2018. ISBN: 9781138359338. URL: <https://bookdown.org/yihui/rmarkdown>.
- [43] Yihui Xie, Christophe Dervieux, and Emily Riederer. *R Markdown Cookbook*. Boca Raton, Florida: Chapman and Hall/CRC, 2020. ISBN: 9780367563837. URL: <https://bookdown.org/yihui/rmarkdown-cookbook>.
- [44] Achim Zeileis and Torsten Hothorn. “Diagnostic Checking in Regression Relationships”. In: *R News* 2.3 (2002), pp. 7–10. URL: <https://CRAN.R-project.org/doc/Rnews/>.