**Between strain analysis**

Differential expression was performed between the four strains A, B, E and I for the following:

1. Non-exercised mice *(File: between-strain-nonex.xlsx)*
2. Exercised responders + exercised non-responders *(File: between-strain-ex.xlsx)*
3. Exercised responders (only for QC) *(File: between-strain-exres.xlsx)*
4. Exercised non-responders (only for QC) *(File: between-strain-exnonres.xlsx)*

We normalized the raw counts to log normalized values using limma + voom. Highly variable and expressed genes were selected using a number of stringent cutoffs: first by expression value cutoff of at least 10 counts in at least 70% of the samples and then by filtering low variance genes using top 50% genes with highest IQR (i.e. top 50% genes with max variability). Using these cutoffs, we reduced the gene list to ~10-15% of the initial transcriptome. For all four analysis, we saw differences between A vs B, A vs E and A vs I strains but no differences were found between any combination of B, E and I strains. This is also seen from the PCA where stain A is clustered separately and B, E and I strains are clustered together. The separation between strain A and the other three strains is mostly determined by principal component 2 (PC2). You can also see separation between the strains based on PC3: do you think this is correlated with any variable that is missing in the excel file?

For all analyses, most differences were between ANT1\_ME vs B6\_ME mice but we also found differences between B6\_ME and IA1 strain for non-exercised mice (adj. Pvalue < 0.05).

**Within strain analysis**

Differential expression was performed within each strain A, B, E and I for the following:

1. Exercised (responders + non-responders) vs Non-exercised *(File: within-strain-ex-vs-nonex.xlsx)*
2. Exercised responders vs Exercised non-responders *(File: within-strain-exres-vs-exnonres.xlsx)*

No significant differences were found for any of the strains. We performed the same steps of normalization and low expression filters as above. For both analyses per strain, PC1 or PC2 were not able to discriminate between exercised vs non-exercised or exercised-responders vs exercised non-responders. So, we generated a PCA plot with PC3 and PC4 to see if those components are responsible to distinguish between the different types of mice. Even those are not able to distinguish between the two groups. There seems to be another variable determining the separation.

**All strains**

Differential expression was performed between:

1. Exercised (responders) vs Exercised (non-responders) of all strains together (*File: all-strains-exres-vs-exnonres.xlsx*)

No significant difference was found. Principal component 2 (PC2) seems to separate the A strain from other strains but no component was found to separate the exercised responders from non-responders.

Adding more stringent filtering for low expression and high variance and reducing the genes for multiple testing did not improve the results.

**Analysis 04/28/2020**

**Sample correlation heatmap:** qc/sample\_correlation.pdf

**Distances between strains**

Distances between centroids of non-exercised and exercised mice.

**Across strain comparison:**

**Output**: distances/distances-across-strains.xlsx

For non-exercised mice

1. **B is close to E < I < A**
2. E is close to I < B < A
3. I is close to E < B < A

For exercised mice

1. **B is close to I < E < A**
2. E is close to I < B < A
3. I is close to E < B < A

Conclusion: B strains were close to E strains in nonexercised but become closer to I strain in exercised mice.

We also combined the exercised and non-exercised mice to calculate the distances and observed that combining the groups or testing them individually makes no difference to the output. Because our data is paired and has an n < 30, we did a Shapiro-Wilk normality test followed by a Wilcoxon Test to check the significance between the groups and got a p-value of 0.0022 which is less than 0.05 and therefore, significant.

**Between strain comparison (05/28/2020):**

**Output**: distances/distances-between-strains.xlsx

For each strain, we computed distances per sample between exercised and non-exercised and compared if there is a difference. We performed an unpaired t-test as well as a unpaired Wilcoxon Test and found that only B6 ME strain has a significant difference (T-test p-value of 0.001; Wilcoxon Test p-value of 0.003) between exercised and non-exercised mice.

**Volcano plots**

**Output**:

1. volcano/\*.pdf showing p-value vs logFC

**Anova**

Anova across 4 strains in exercised and non-exercised mice. P-values adjusted using Bonferroni. Heatmaps show all genes with adjusted p-value < 0.05.

Based on the reference paper, our methods agree as the authors also use adj. pvalue < 0.05 to create the heatmap.

Anova Heatmaps: anova/\*heatmap.pdf

Anova results as excel format:

1. Exercised mice: exercised-anova.xls
2. Non-exercised mice: non-exercised-anova.xls

Pathway enrichment using DEGs (adj. Pvalue < 0.05) from anova using Metacore for both analysis:

1. Pathway Maps: \*pathwaymaps.xls
2. Process Networks: \*processnetworks.xls
3. GO Processes: \*goprocesses.xls

**Analysis 05/28/2020**

**Metacore analysis for all comparisons**

We used **Metacore GO Processes** on all comparisons where we had differentially expressed genes adjusted p-value < 0.05. We got upregulated and downregulated pathways by splitting the lists into up and down genes by logFC > 1 (for up) and logFC < -1 (for down) except for B6 vs IAI (Downregulated Pathways), where we had to use logFC < 0 because no genes where downregulated with logFC < -1.

**Output**:

1. metacore/

**GSEA**

Exercised vs Non-exercised comparisons for overall matrix and for strain specific matrix. We first converted the gene symbols to human gene symbols and used GSEA for the enrichment (GSEA is designed for human data, by default). Results will be uploaded by end of business.

**Output**:

1. gsea/ contains zipped folders for all five comparisons.

**Covariate Analysis**

Genes seem to be more correlated to strains than by covariates which means we cannot do a per strain correlation because that would just give us genes correlated to strain. Also, VO2max for ANT1 exercised is lower than ANT1 non-exercised which needs to be clarified. Created some boxplots for covariates and strains/group to visualize that. Linear model was done between genes and covariates.

**Output**:

1. covars/.xlsx files are output from the linear model between genes and covariates. P-values were adjusted using FDR. Anything that has FDR < 0.05 was treated as correlated.
2. covars/plots are boxplots for association between strains and covariates.