At the centre of the reviewers' cogent critique of our manuscript was the questioned statistical validity of our approach. Succinctly, the issue at question is whether or not the R package `edgeR` is an appropriate tool for analysis of protein mass spectrometry data.

High level statistical inference in `edgeR` is built on a negative binomial (NB) generalized linear model (GLM) framework. The data are assumed to be adequately described by a NB distribution parameterized by a dispersion parameter, ϕ. [1]

Our previous approach used a customized workflow[2], to preprocessing and normalize the data. We used `edgeR` to perform statistical testing using its flexible GLM framework.
Our decision to use `edgeR` was motivated by numerous conceptual and practical considerations. `edgeR` is an excellent package, and should be strongly considered when analyzing RNA-sequencing data. Here we only consider its appropriatness for our TMT dataset.

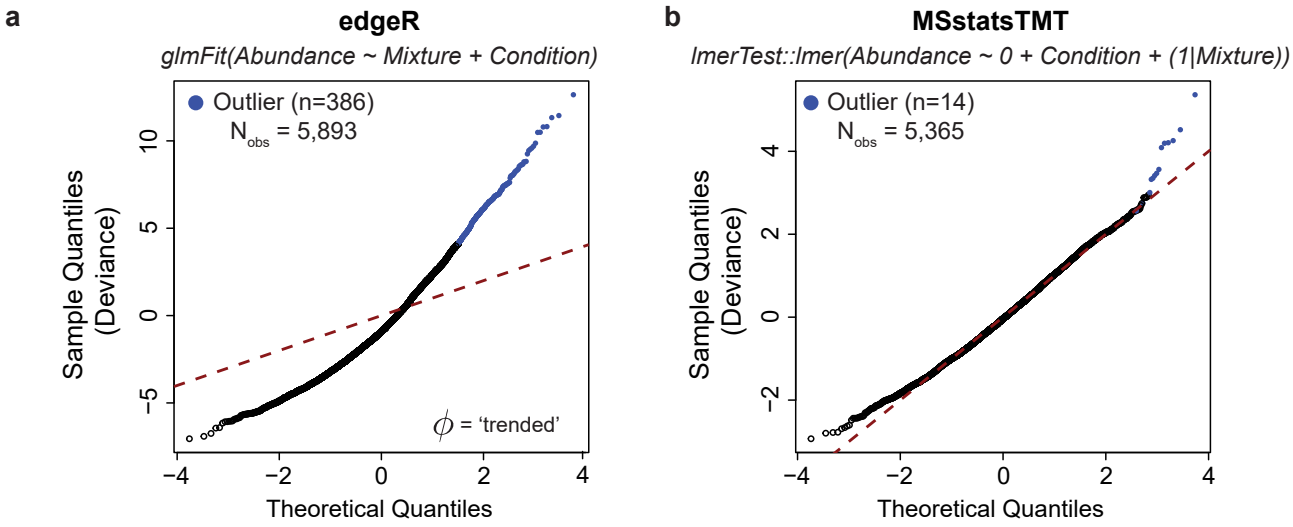

Figure 1: **Goodness-of-fit of `edgeR` (a), and `MSstats` (b) statistical approaches.** (a) The data were fit with `edgeR::glmFit` using 'trended' dispersion. The residual deviance is plotted as a quantile-quantile plot using `edgeR::gof`. (b) The data were fit with a linear mixed model to account for random effect of Mixture.

[1]The dispersion parameter can take several forms. p supports three types of dispersion models: 'common', 'trended', and 'tagwise'. When using `edgeR's` robust quasi-likelihood test methods, only global (i.e. 'common' or 'trended') dispersions are appropriate (see ?`edgeR::glmQLFit`).

[2]The most important step in our normalization approach is IRS normalization. IRS normalization scales protein measurements using an internal reference standard to normalize protein measurments between TMT MS runs. This is essential to account for the stochasticisity of peptide quantification in MS experiments. Phillip Wilmarth's GitHub offers an excellent exploration of IRS normalization.