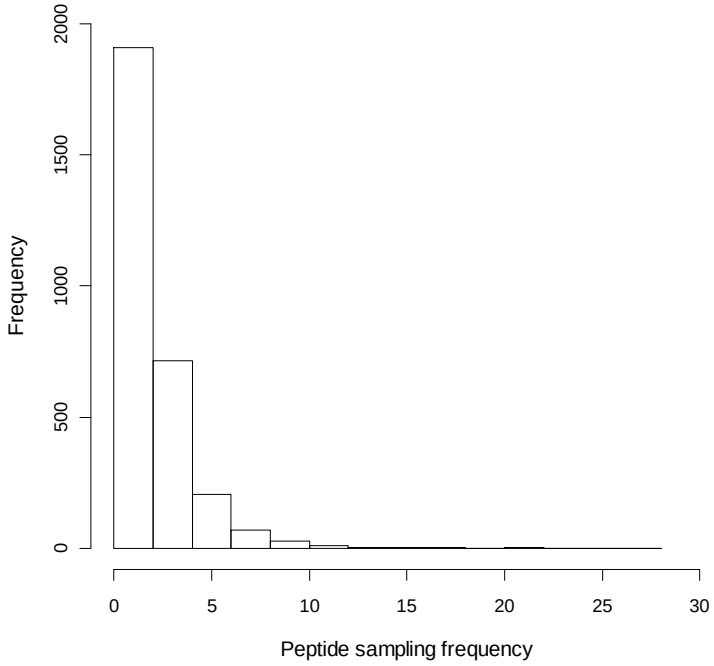
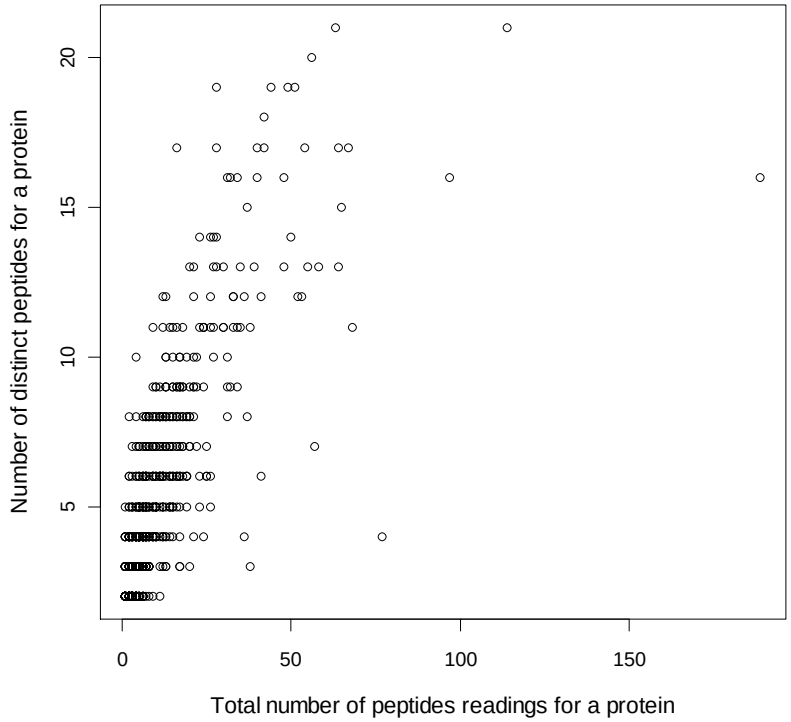
Supplementary figures

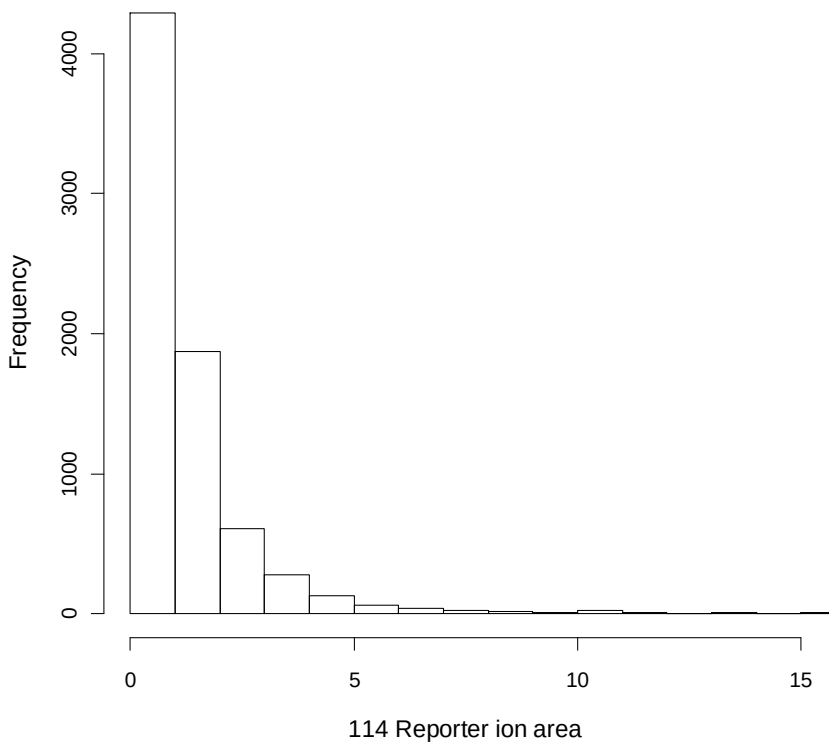


A

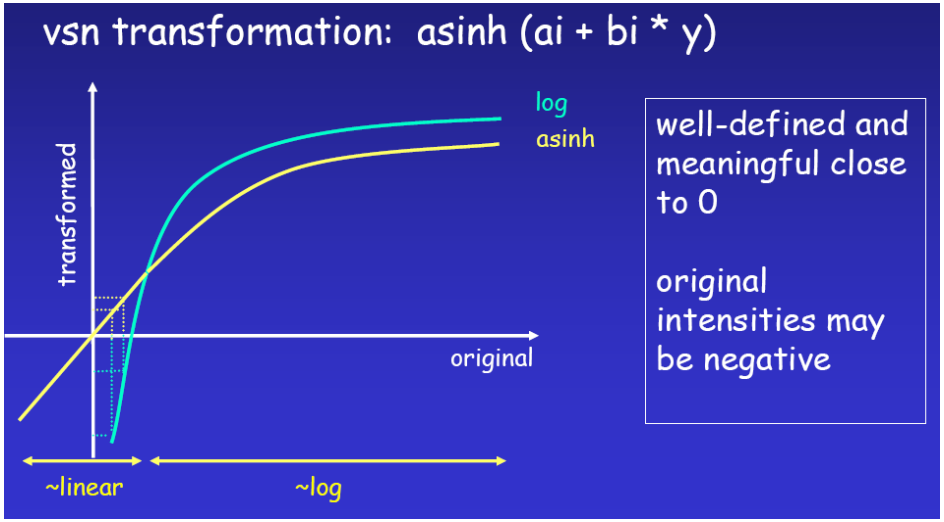


B

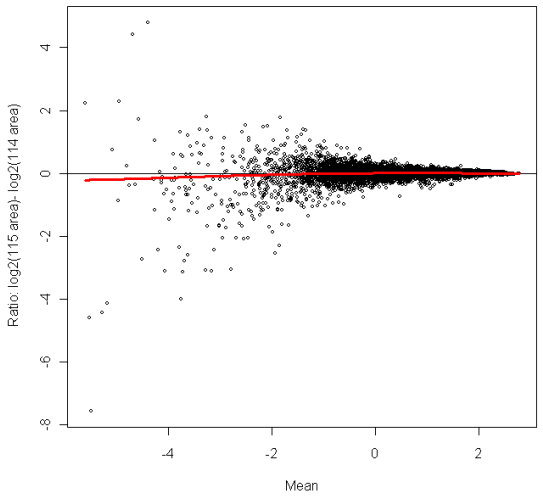
Supplementary figure 1: iTRAQ quantitation suffers from unbalanced peptide sampling. A: Frequency histogram of the number of times a peptide is sampled over the course of a single iTRAQ experiment. B: Comparison of the total number of peptides used for quantitation compared to the number of unique peptides for that protein. Data shown is from *Erwinia* same-same dataset B.



Supplementary figure 2: Frequency histogram of 114 reporter ion area for *Erwinia* dataset B demonstrating how low volume signals predominate. For clarity the x-axis displayed has been limited from a range of 0 to 40 to 0 to 15.



Supplementary figure 3: Graphical representation of the effect of a log and asinh transformation.



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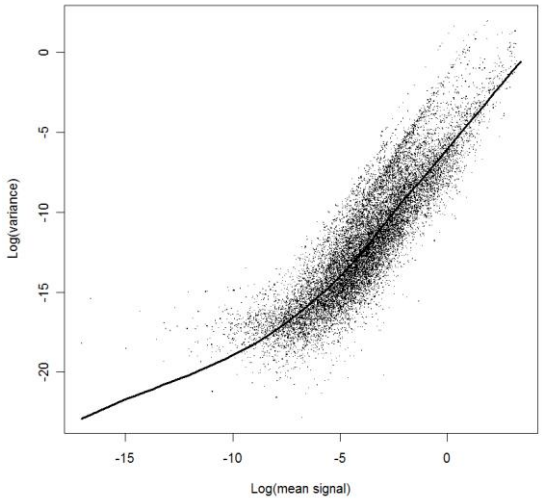

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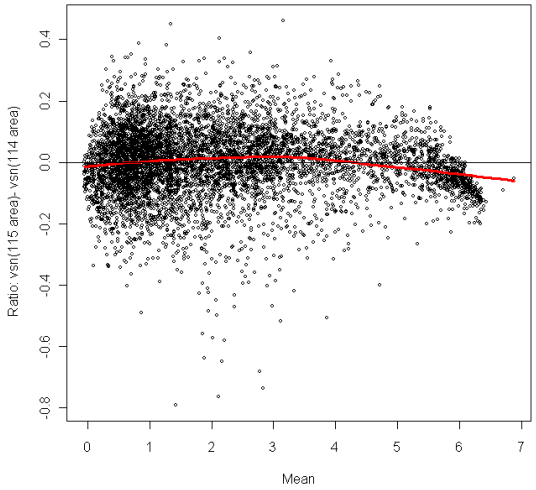

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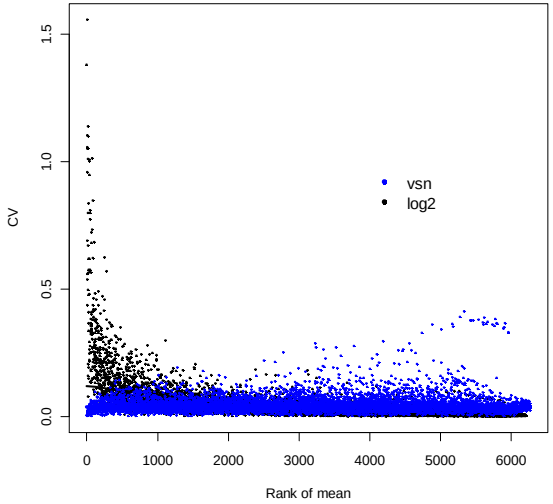
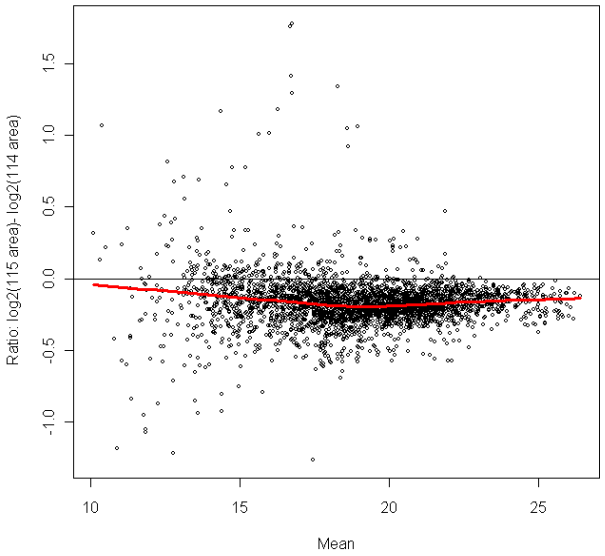


D

Supplementary figure 4: figures to highlight peptide data behaviour seen for the phosphorylase B 4-plex QSTAR data when Mascot was used for quantitation. A: Ratio-intensity plot for log2 data, B: The relationship between the logarithm (base 2) of the mean signal and the logarithm (base 2) of its variance. C: Ratio-intensity plot for VSN transformed data, and D: CV comparison for log2 versus VSN transformed data.



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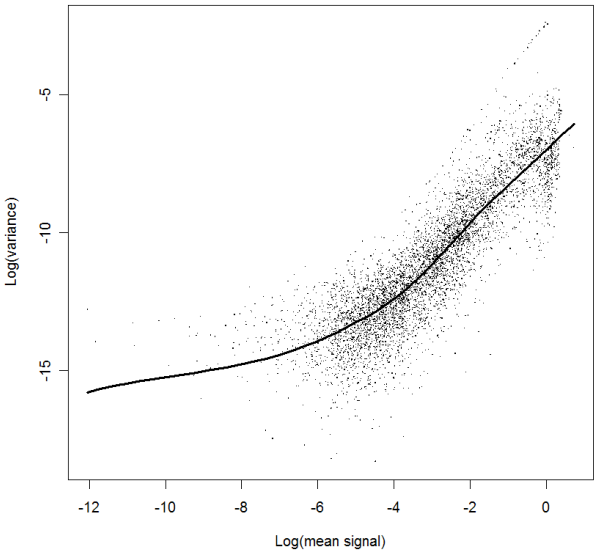

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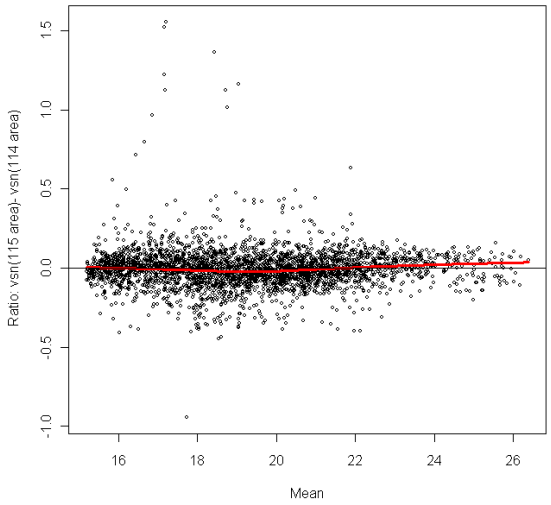

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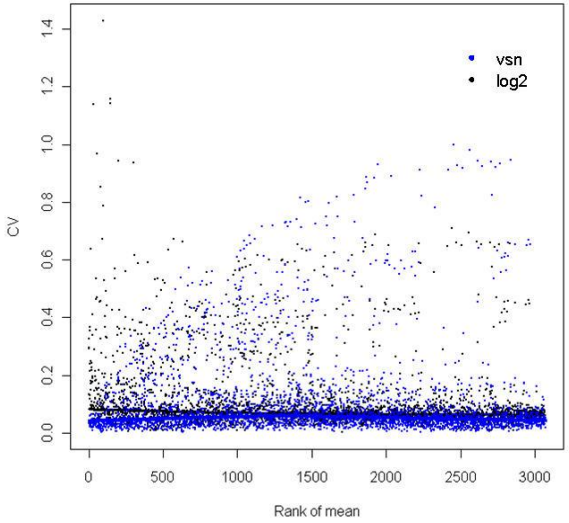
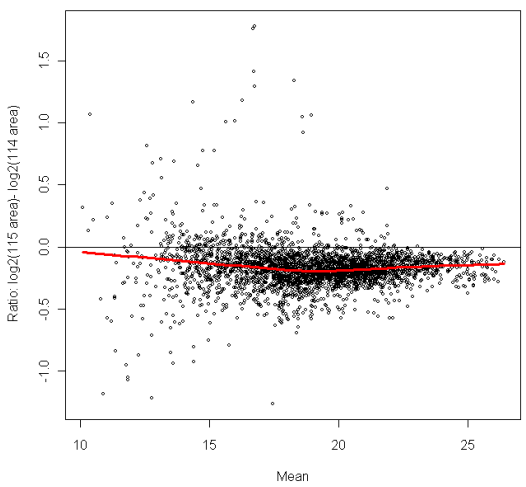


D

Supplementary figure 5: figures to highlight peptide data behaviour seen for the phosphorylase B 4-plex Orbitrap XL data when Proteome Discoverer was used for quantitation. A: Ratio-intensity plot for log2 data, B: The relationship between the logarithm (base 2) of the mean signal and the logarithm (base 2) of its variance. C: Ratio-intensity plot for VSN transformed data, and D: CV comparison for log2 versus VSN transformed data.



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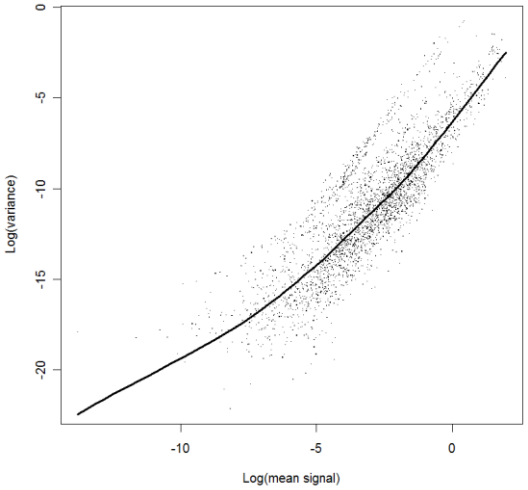

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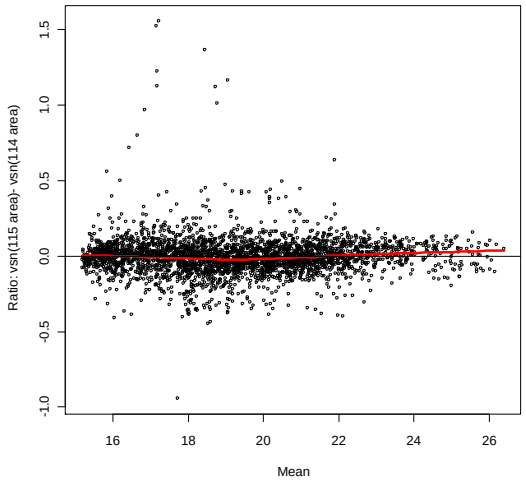

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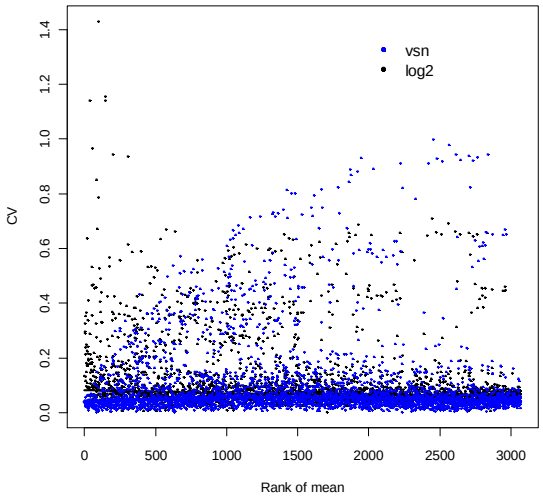
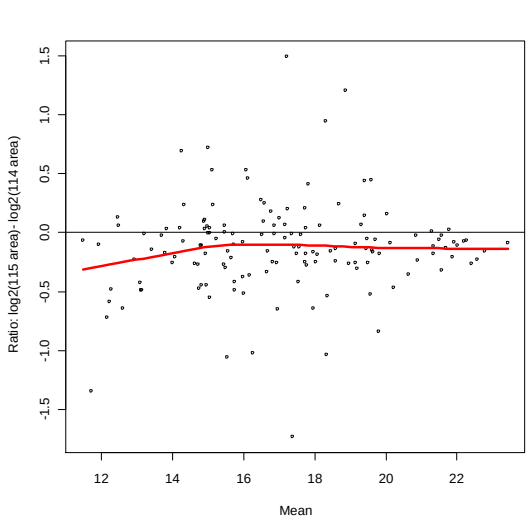


D:

Supplementary figure 6: figures to highlight peptide data behaviour seen for the 4-plex phosphorylase B dataset obtained from repeat injection of same-same phosphorylase B into an OrbitrapXL system and the signal quantified with MASCOT. A: Ratio-intensity plot for raw data, B: The relationship between the logarithm (base 2) of the mean signal and the logarithm (base 2) of its variance, C: Ratio-intensity plot for VSN transformed data, and D: CV comparison for log2 versus VSN transformed data.



A

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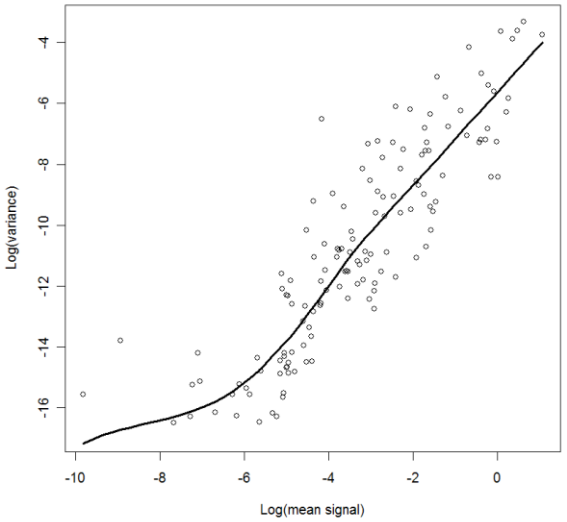

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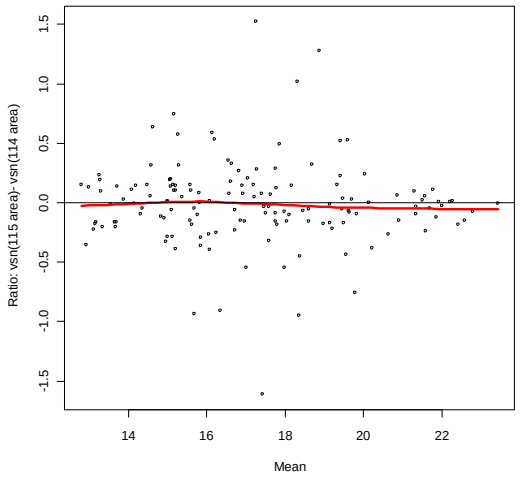

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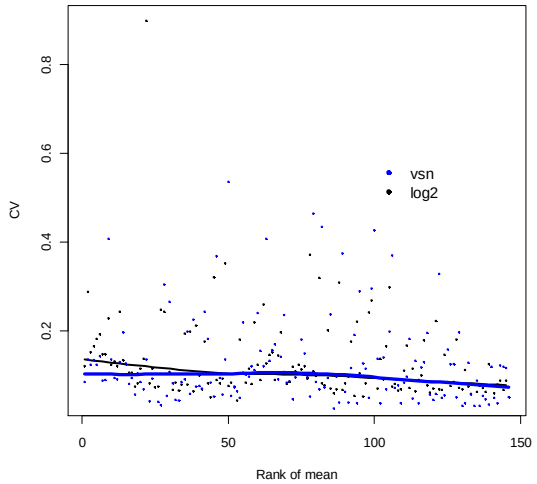
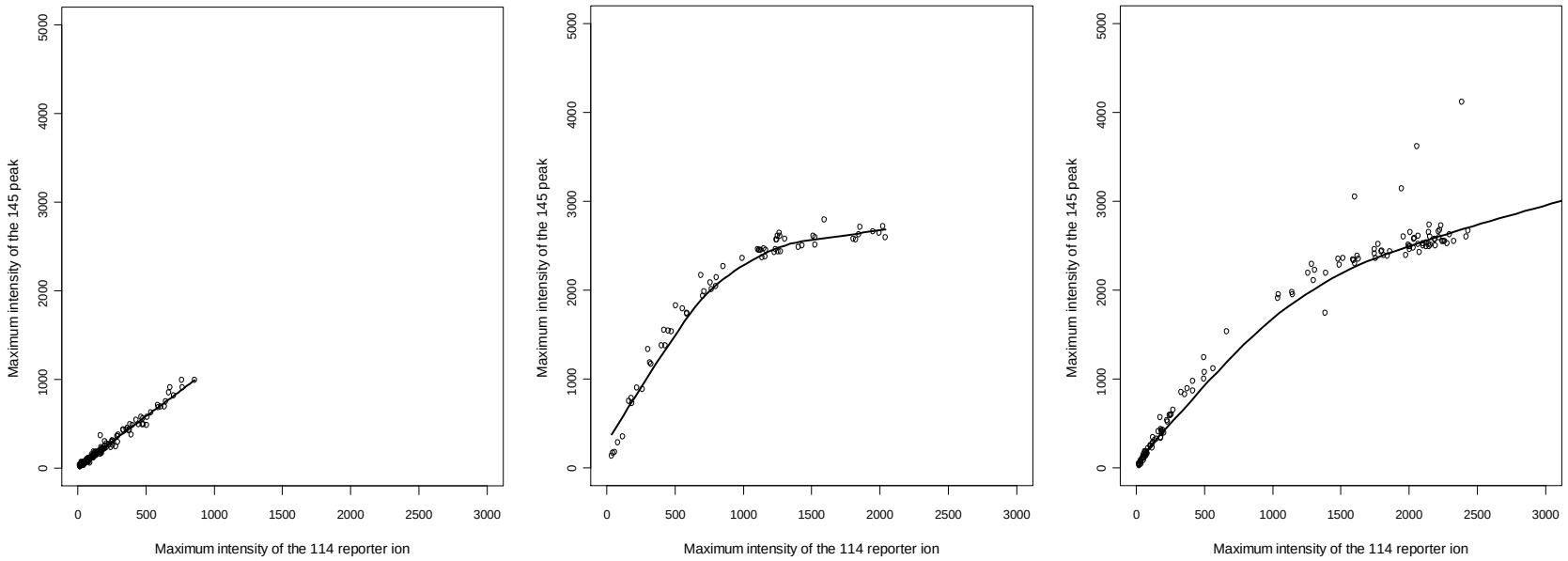


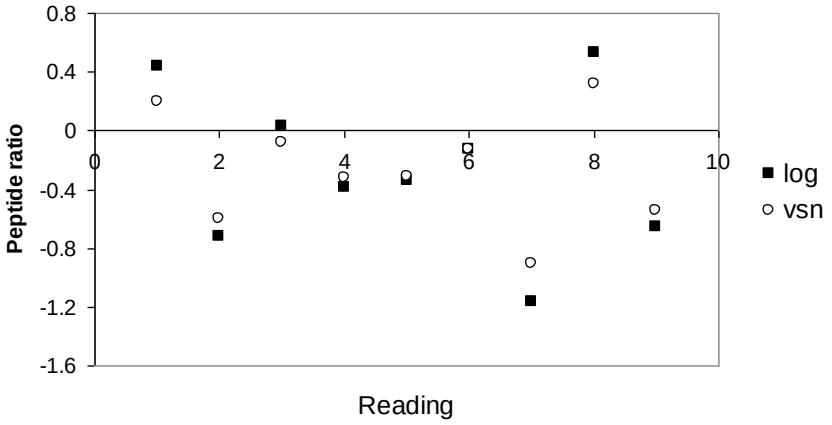
D:

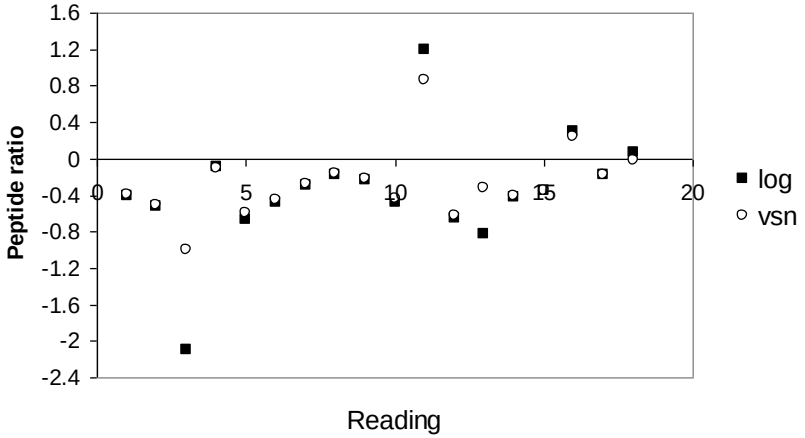
Supplementary figure 7: figures to highlight peptide data behaviour seen for the 8-plex phosphorylase B dataset obtained from repeat injection of same-same phosphorylase B into an OrbitrapXL system and the signal quantified with MASCOT. A: Ratio-intensity plot for raw data, B: means-variance plot for raw data, C: Ratio-intensity plot for VSN transformed data, and D: CV comparison for raw versus VSN transformed data.



A B C

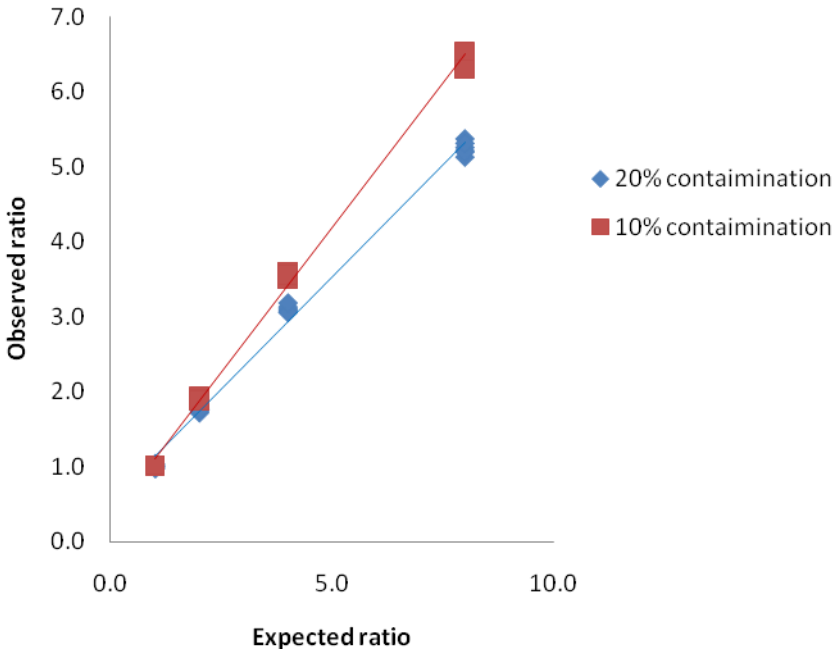
Supplementary figure 8: Examples of the variation in fragmentation behaviour seen for three different peptides by examining the relationship between the 114 reporter ion and the 145 peak measurements for that peptide. A: Peptide with linear behaviour across the intensity range sampled. B: Peptide with a non-linear behaviour, where the amount of un-fragmented peak decreased relative to the reporter ion. C: Example of variable behaviour, with the majority of measurements for this peptide giving a non-linear behaviour at the high intensity readings.



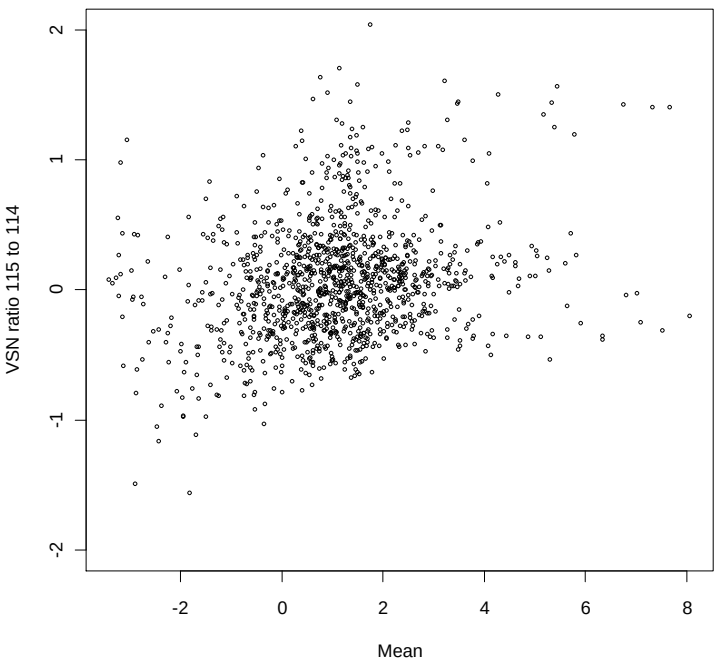


A B

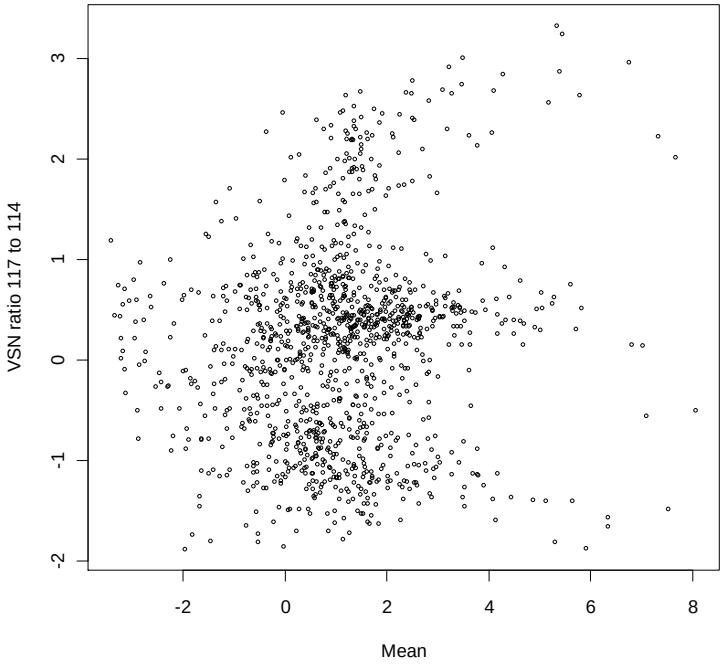
Supplementary figure 9: Comparison of the peptide readings for two proteins which were significant with the VSN transformed data but not the log transformed data within the yeast study. A: Peptide readings for protein YDL029W and B: peptide readings for protein YELO6OC.



Supplementary figure 10: Data modelling was used to investigate the impact of precursor ion contaimnation during an experiment. Two different levels of contaimnation were considered and the resulting behaviour compared. This analysis assumes the contamination remains constant within an experiment and that the peptides selected on average have a 1 to 1 ratio across the isobaric tags.

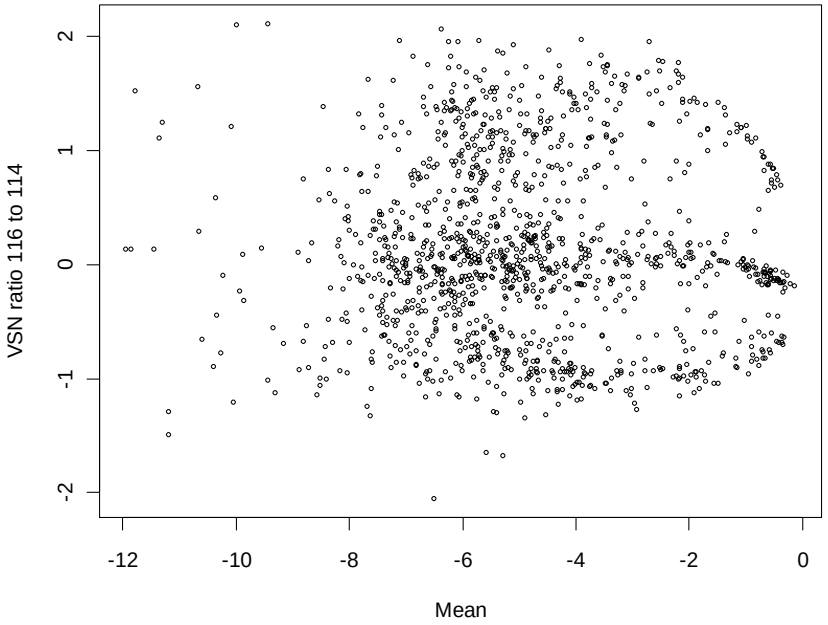


A

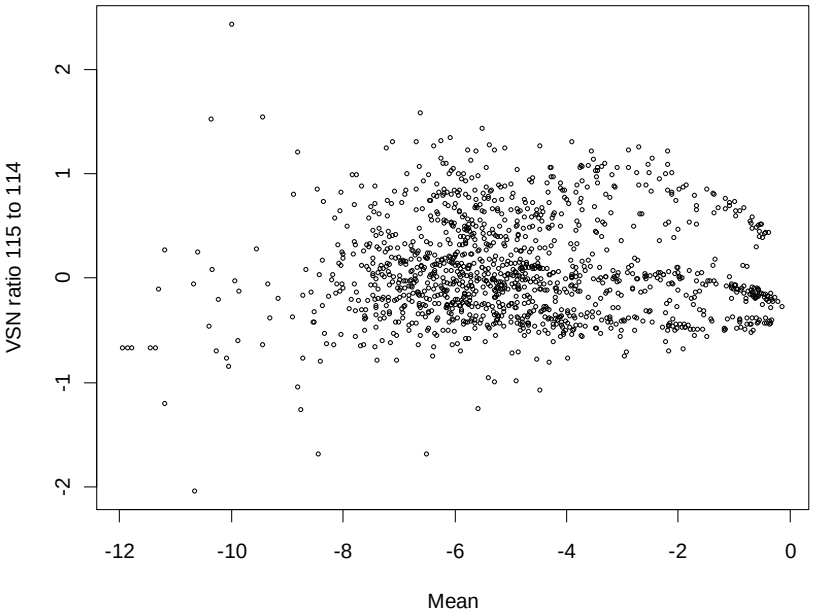


B

Supplementary figure 11: Ratio-Intensity plot at the peptide level for the known ratio study completed on the QToF Premier. A: Shows the ratio behaviour seen between isobaric tags 116 and 114. B: Shows the ratio behaviour seen between isobaric tags 115 to 114.



A



B

Supplementary figure 12: Ratio-Intensity plot at the peptide level for the known ratio study completed on the Q-STAR. A: Shows the ratio behaviour seen between isobaric tags 116 and 114. B: Shows the ratio behaviour seen between isobaric tags 115 to 114.