

Figure S1

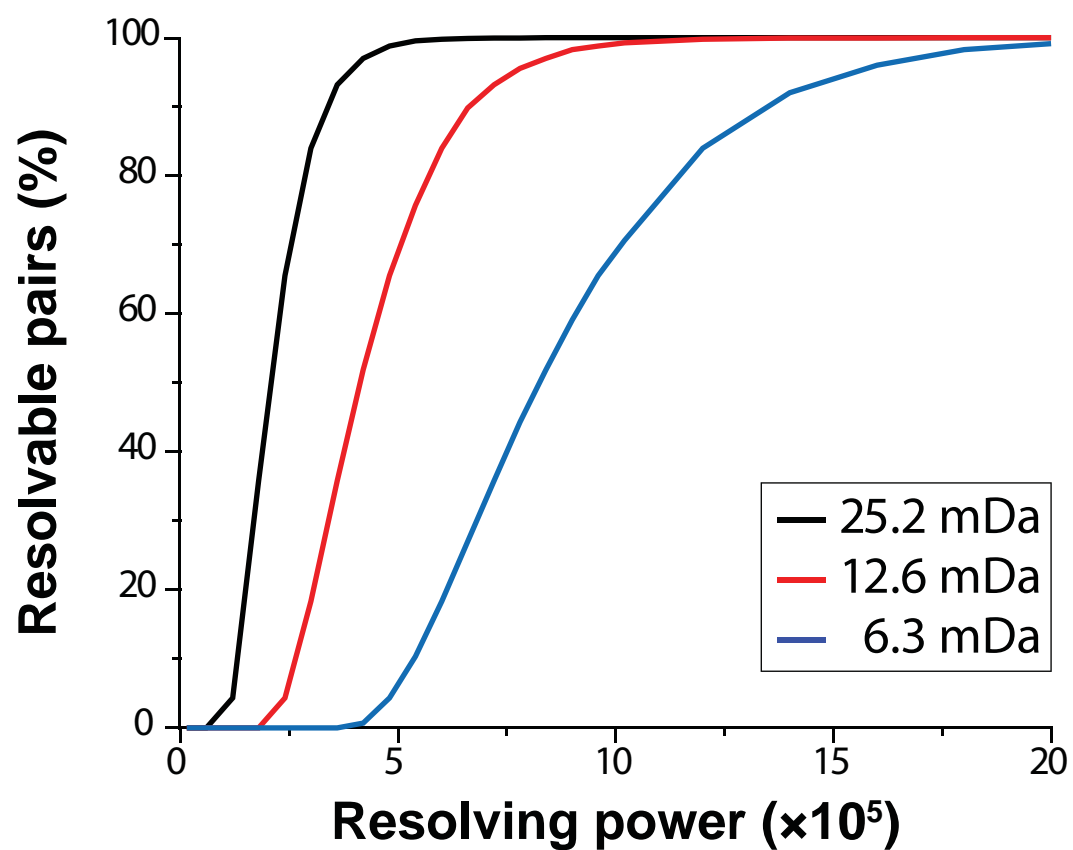


Figure S1 | NeuCode amine-reactive label design. a, Theoretical calculation depicting the percentage of peptides that are resolved at full-width at 10% maximum peak height (FWTM) when spaced 6.3, 12.6, or 25.2 mDa for resolving powers 15 thousand to 2 million on an FT-ICR.

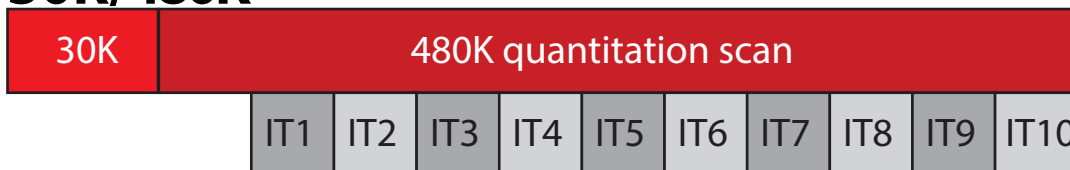
Figure S2

a

30K



30K/480K



b

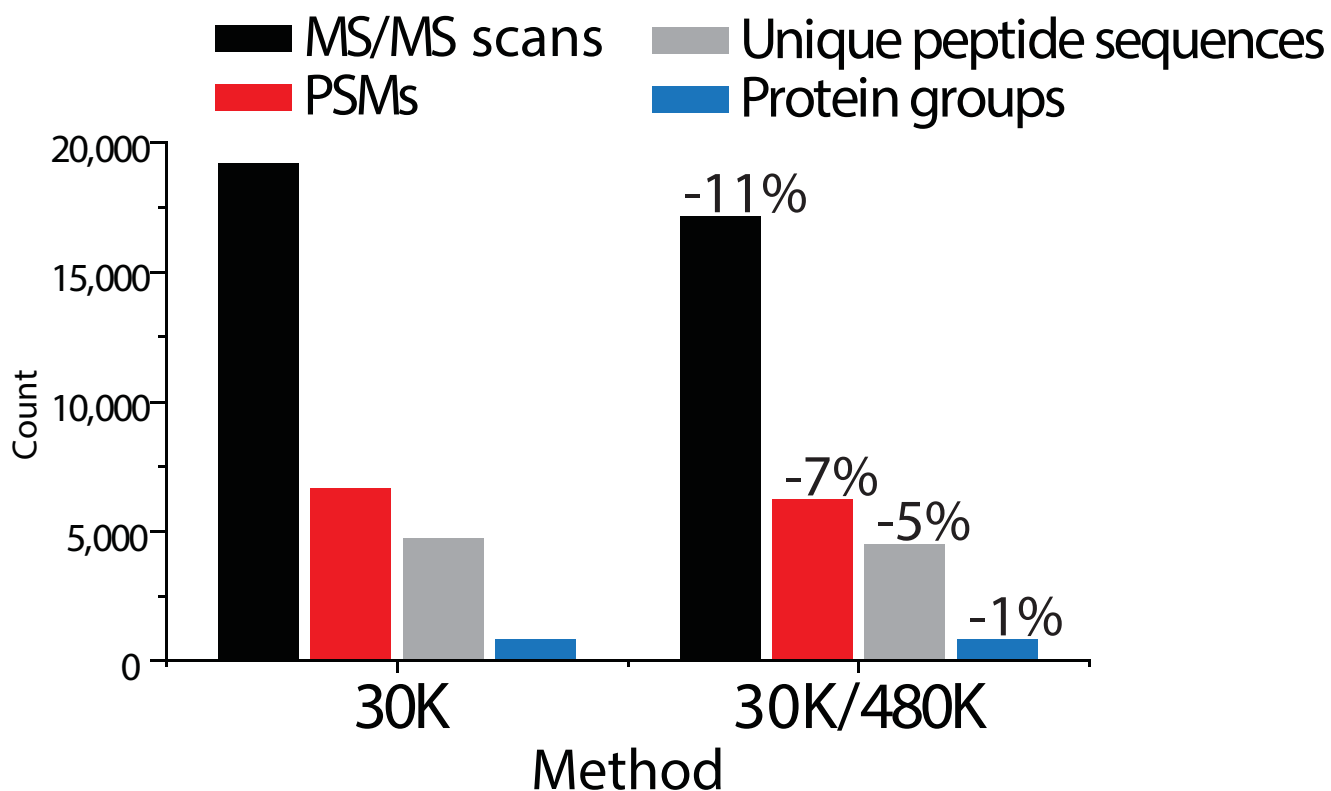


Figure S2 | Effect of 480K scan on duty cycle. A yeast tryptic digest LC-MS/MS analysis, with a 60 min gradient elution, using a method with a 480,000 resolving power scan and one with only a 30,000 resolving power scan. **a**, Approximate scans sequence order. **b**, Duty cycle metrics from analyses with and without a 480K scan.

Figure S3

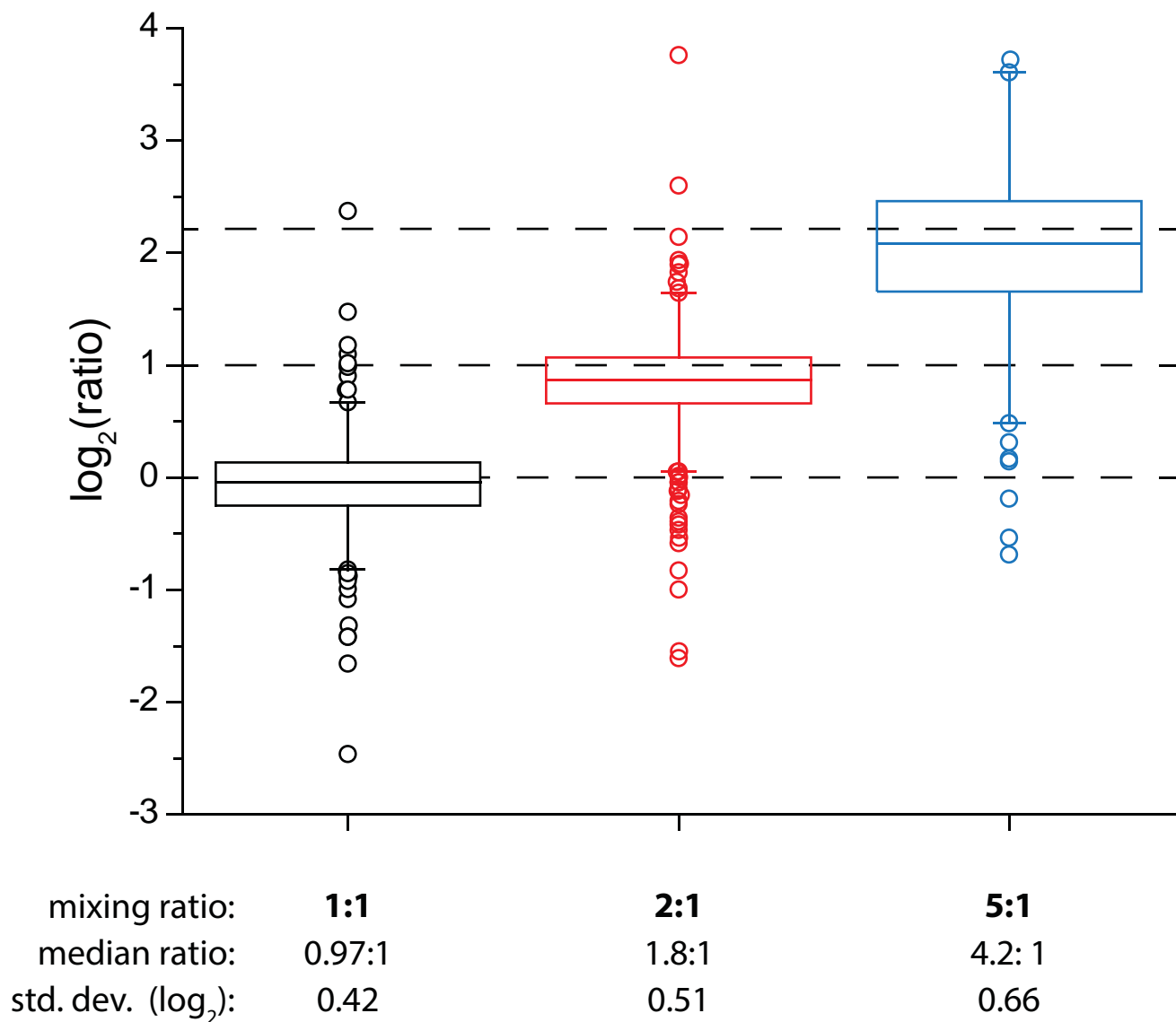


Figure S3 | Boxplot of measured protein ratios. A yeast tryptic digest was split into 4 equivalent aliquots, labeled independently with a NeuCode 4-plex tag, and mixed in the ratio 1:10:5:2. Measured 1:1, 2:1, and 5:1 ratios of proteins are represented as a box plot. The median ratios and standard deviations (std. dev.) are shown for each ratio, below the plot. Expected ratios are denoted as a dashed line.

Figure S4

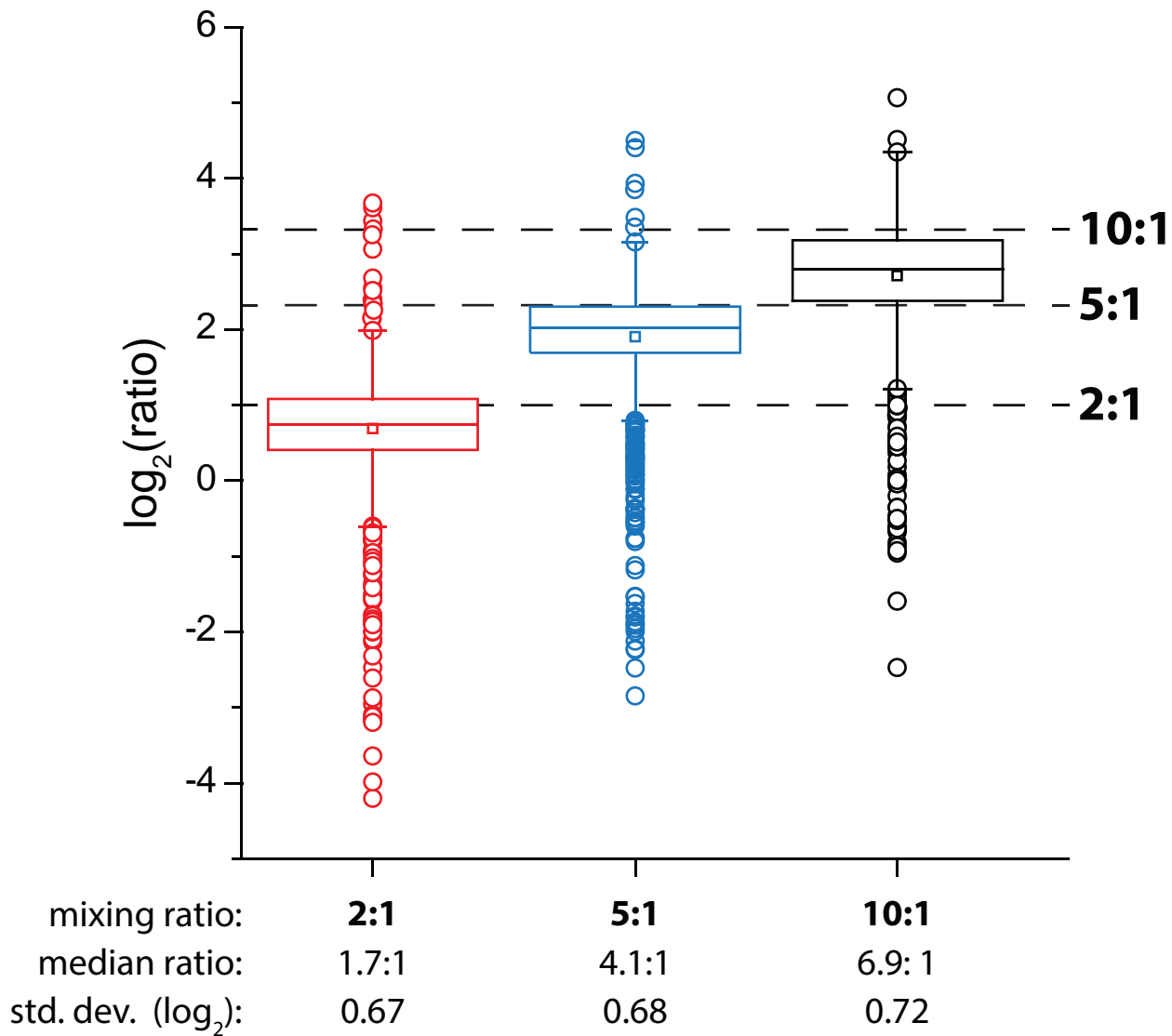
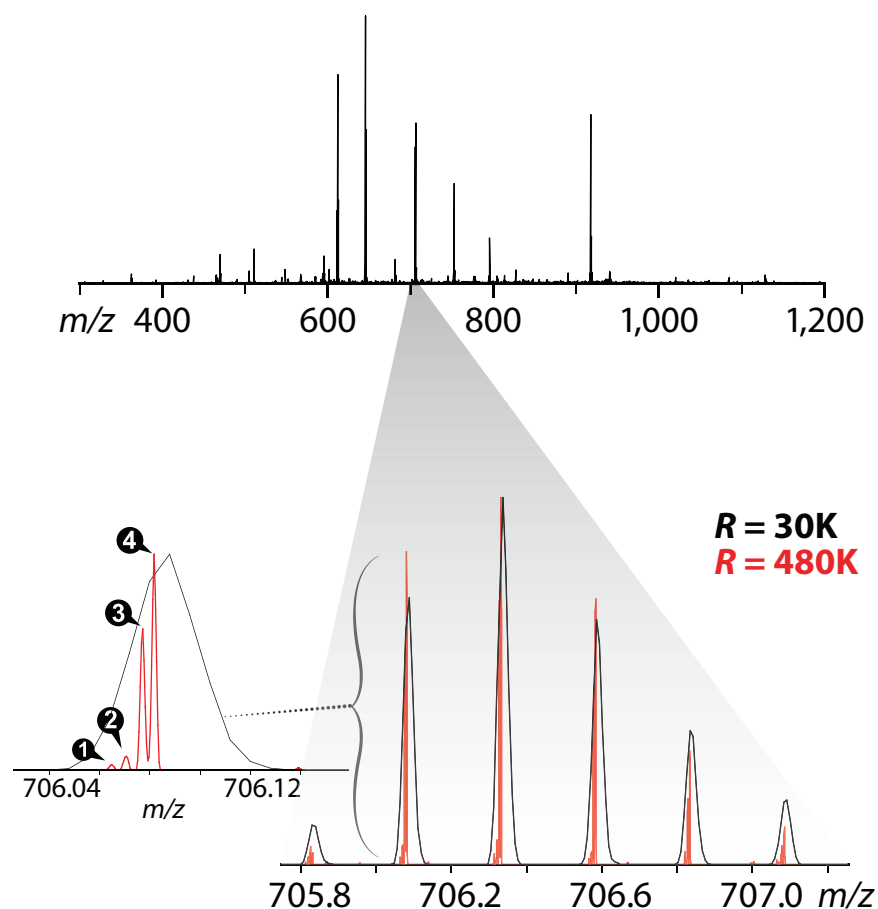


Figure S4 | Boxplot of measured ratios. A yeast tryptic digest was split into 4 equivalent aliquots, labeled independently with a NeuCode 4-plex tag, and mixed in the ratio 1:10:5:2. Measured 2:1, 5:1, and 10:1 ratios of peptides are represented as a box plot. The median ratios and standard deviations (std. dev.) are shown for each ratio, below the plot. Expected ratios are denoted as a dashed line.

Figure S5

a



b

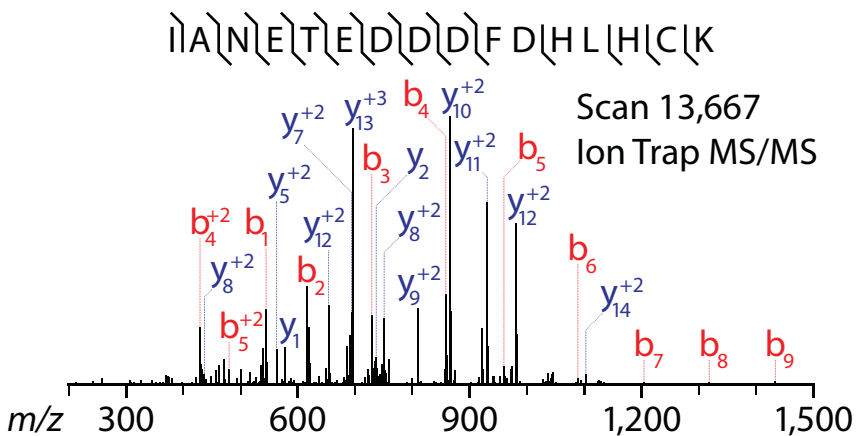


Figure S5 | MS1 scan collected from a nanoHPLC-MS/MS analysis of tryptic yeast diauxic peptides that had been labeled with four-plex NeuCode tags. The inset displays the isotopic distribution of a precursor at m/z 706 at 30,000 resolving power (black) and at 480,000 resolving power (red). The monoisotopic peak is further expanded to display the embedded quantitative information. Note there is an M-1 peak present, from isotopic impurities. c, Annotated MS2 spectrum and sequence, of the peptide from b, following CAD and ion trap m/z analysis. The peptide shown here is derived from the protein RG11 which is known to increase in abundance under aerobic conditions.

Figure S6

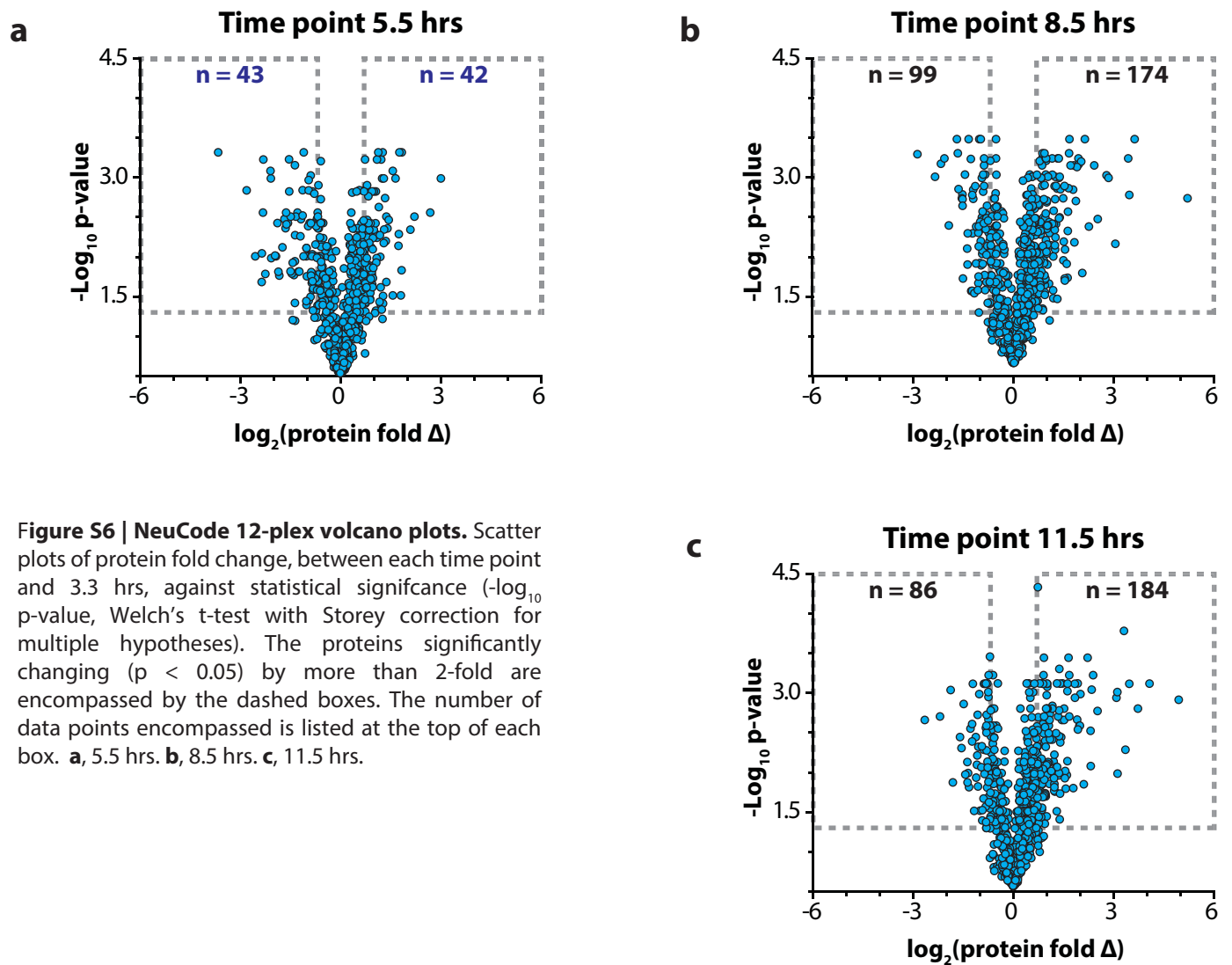


Figure S6 | NeuCode 12-plex volcano plots. Scatter plots of protein fold change, between each time point and 3.3 hrs, against statistical significance ($-\log_{10}$ p-value, Welch's t-test with Storey correction for multiple hypotheses). The proteins significantly changing ($p < 0.05$) by more than 2-fold are encompassed by the dashed boxes. The number of data points encompassed is listed at the top of each box. **a**, 5.5 hrs. **b**, 8.5 hrs. **c**, 11.5 hrs.