

Supplementary data for

Optimising Spectronaut search parameters to improve data quality with minimal proteome coverage reductions in DIA analyses of heterogeneous samples

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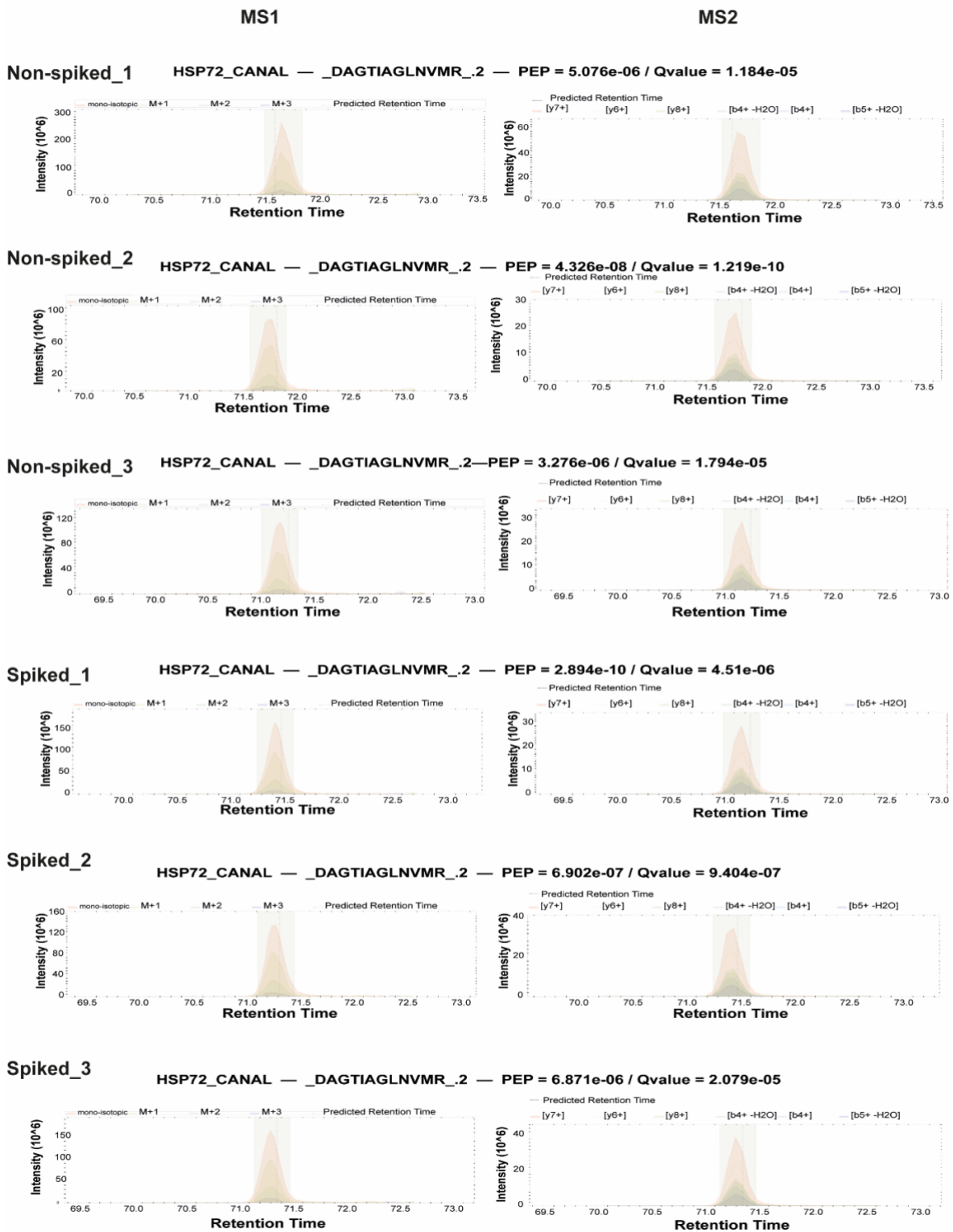
Supplemental Figure 2: XIC for DAGTIAGLNVMR_.2 with stringent settings

Supplemental Figure 3: XIC for AADETISWLDANQTATQEEFADQKELESK_.3

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Supplemental Figure 5: Significantly changed proteins across the different Spectronaut settings

HSP72_CANAL_DAGTIAGLNVMR_2 Standard settings



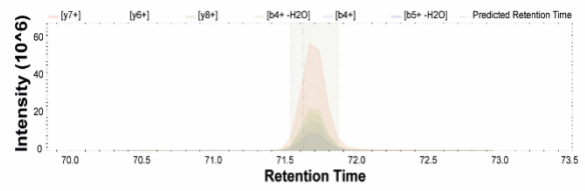
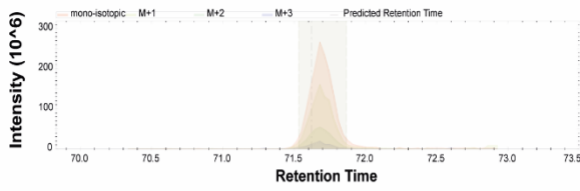
Supplementary Figure 1: Spectronaut 17 default settings MSI and MS2 extracted ion chromatogram (XIC) profiles for *C. albicans* protein HSP72 and precursor DAGTIAGLNVMR_2, across all samples, both spiked and non-spiked.

HSP72_DAGTIAGLNVMR_2_Stringent settings
MS1

MS2

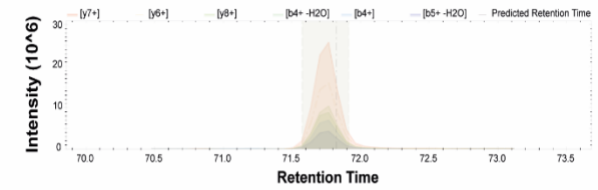
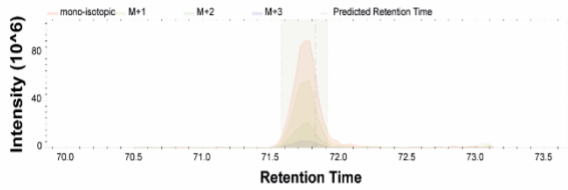
Non-spiked_1

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 5.076e-06 / Qvalue = 1.184e-05



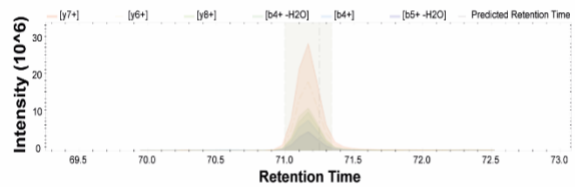
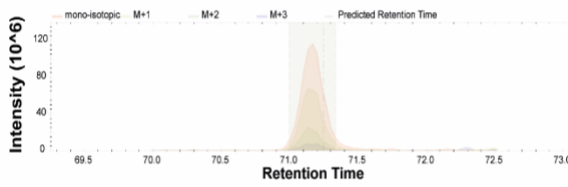
Non-spiked_2

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 4.326e-08 / Qvalue = 1.219e-10



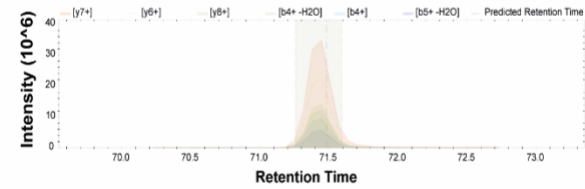
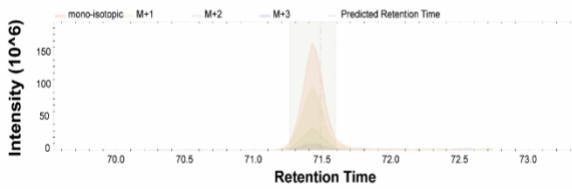
Non-spiked_3

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 3.276e-06 / Qvalue = 1.794e-05



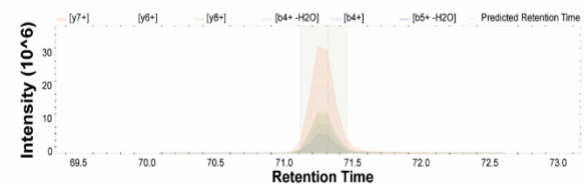
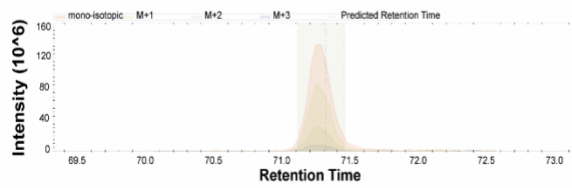
Spiked_1

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 2.894e-10 / Qvalue = 4.51e-06



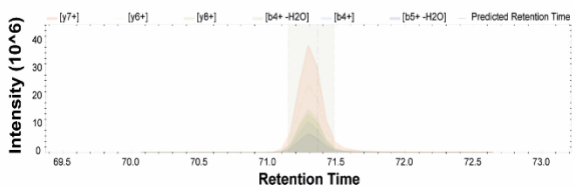
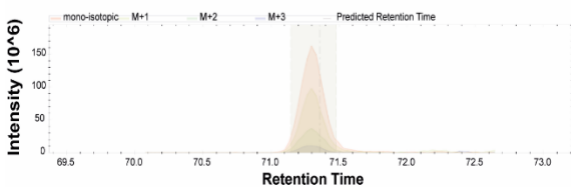
Spiked_2

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 6.902e-07 / Qvalue = 9.404e-07



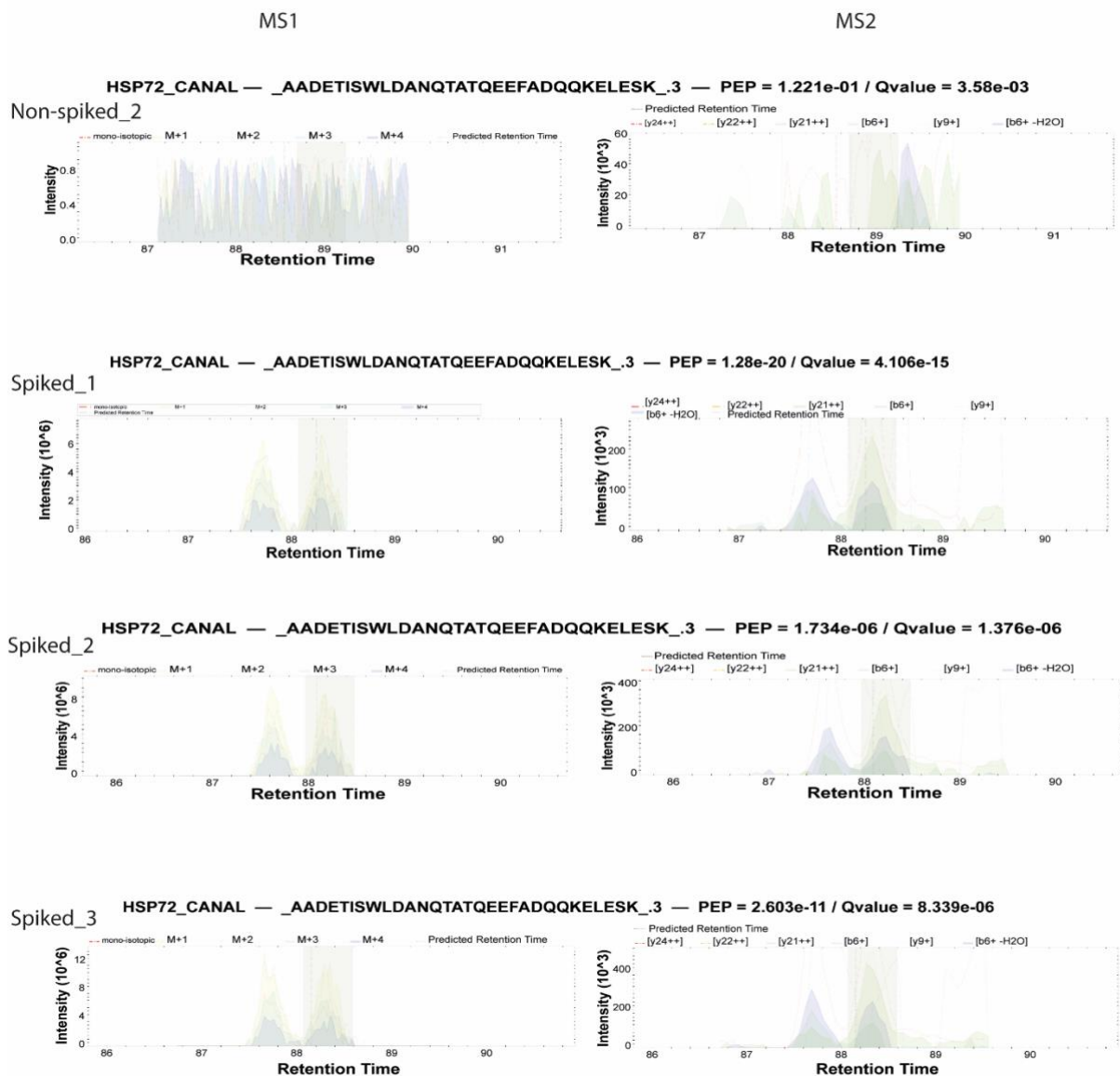
Spiked_3

HSP72_CANAL — _DAGTIAGLNVMR_2 — PEP = 6.871e-06 / Qvalue = 2.079e-05



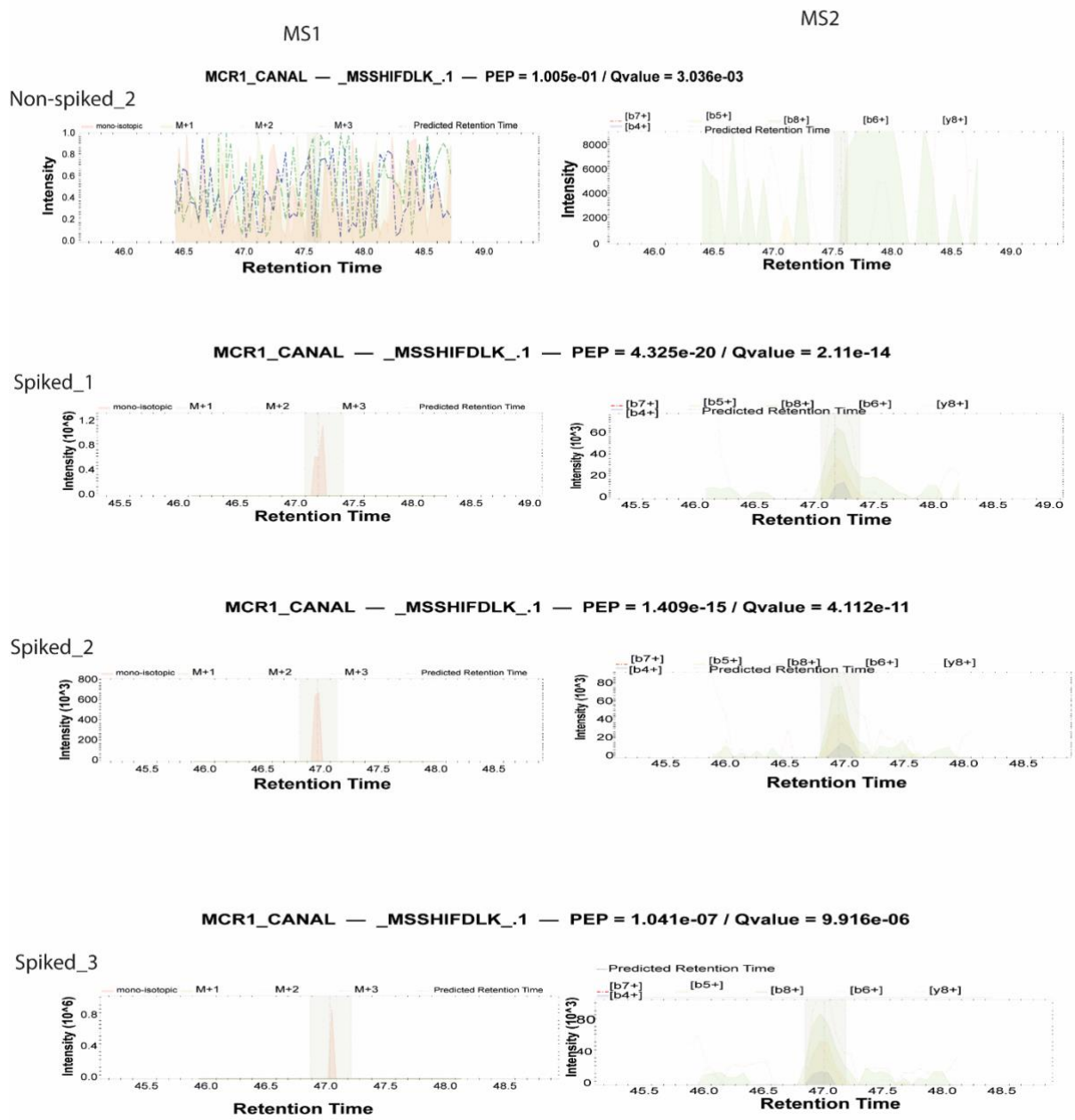
Supplementary Figure 2: Spectronaut 17 stringent settings MSI and MS2 extracted ion chromatogram (XIC) profiles for *C. albicans* protein HSP72 and precursor DAGTIAGLNVMR_2, across all samples, both spiked and non-spiked.

HSP72_AADETISWLDANQTATQEEFADQQKELESK_3

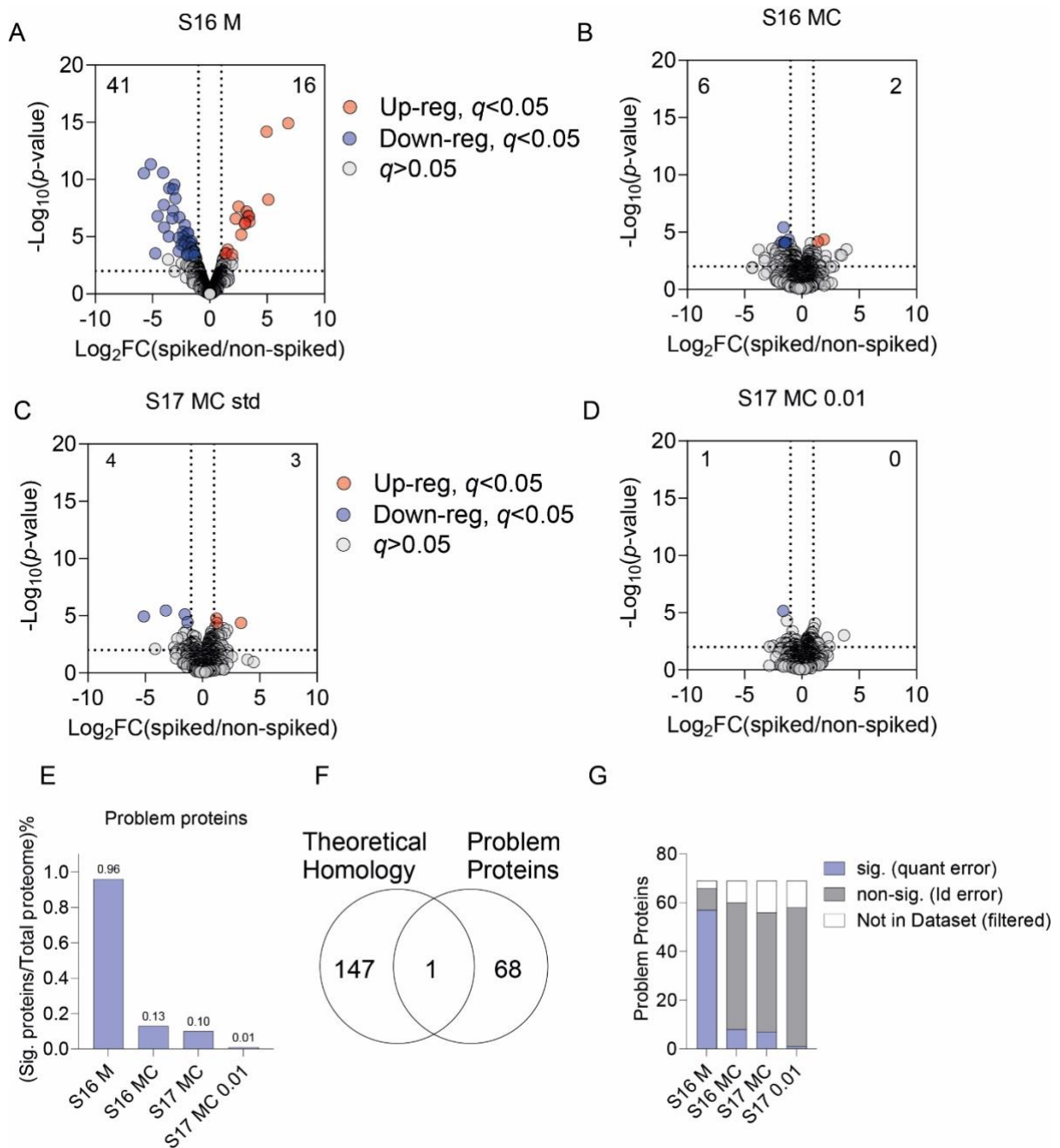


Supplementary Figure 3: Spectronaut 17 default settings MSI and MS2 extracted ion chromatogram (XIC) profiles for *C. albicans* protein HSP72 and precursor AADETISWLDANQTATQEEFADQQKELESK_3, across all samples, both spiked and non-spiked.

MCR1_Canal__MSSHIFDLK_1



Supplementary Figure 4: Spectronaut 17 default settings MSI and MS2 extracted ion chromatogram (XIC) profiles for *C. albicans* protein HSP72 and precursor MSSHIFDLK_1, across all samples, both spiked and non-spiked.



Supplementary Figure 5. Volcano plots for (A) Spectronaut 16, default settings with a Mouse only FASTA file, and (B) Spectronaut 16, default settings (C) Spectronaut 17, default settings and (D) Spectronaut 17 with 0.01 identification settings with a Mouse FASTA and a Candida FASTA. (D-E) The significant proteins from each analysis were pooled to make a compiled list of "problematic proteins" q -value < 0.05 and fold change > 2 . (E) Plots problematic proteins in each analysis as a percentage of the total proteins identified in the dataset. (F) compares this list of Mouse problematic proteins against the theoretical homology between Mouse and *C. albicans* to determine how many of these errors are due to homology or misidentification while (G) plots the problematic proteins, highlighting the number of significant or non-significant proteins for that analysis, as well as proteins that were not identified in the analysis. Volcano plots show the Log_2 fold change (spiked vs non-spiked), and the $-\text{Log}_{10}$ p-value and highlights proteins with a p-value < 0.01 and $-2 > \text{FC} > 2$.