SUPPORTING MATERIALS FOR

Improving SILAC quantification with data independent acquisition to investigate bortezomib-induced protein degradation

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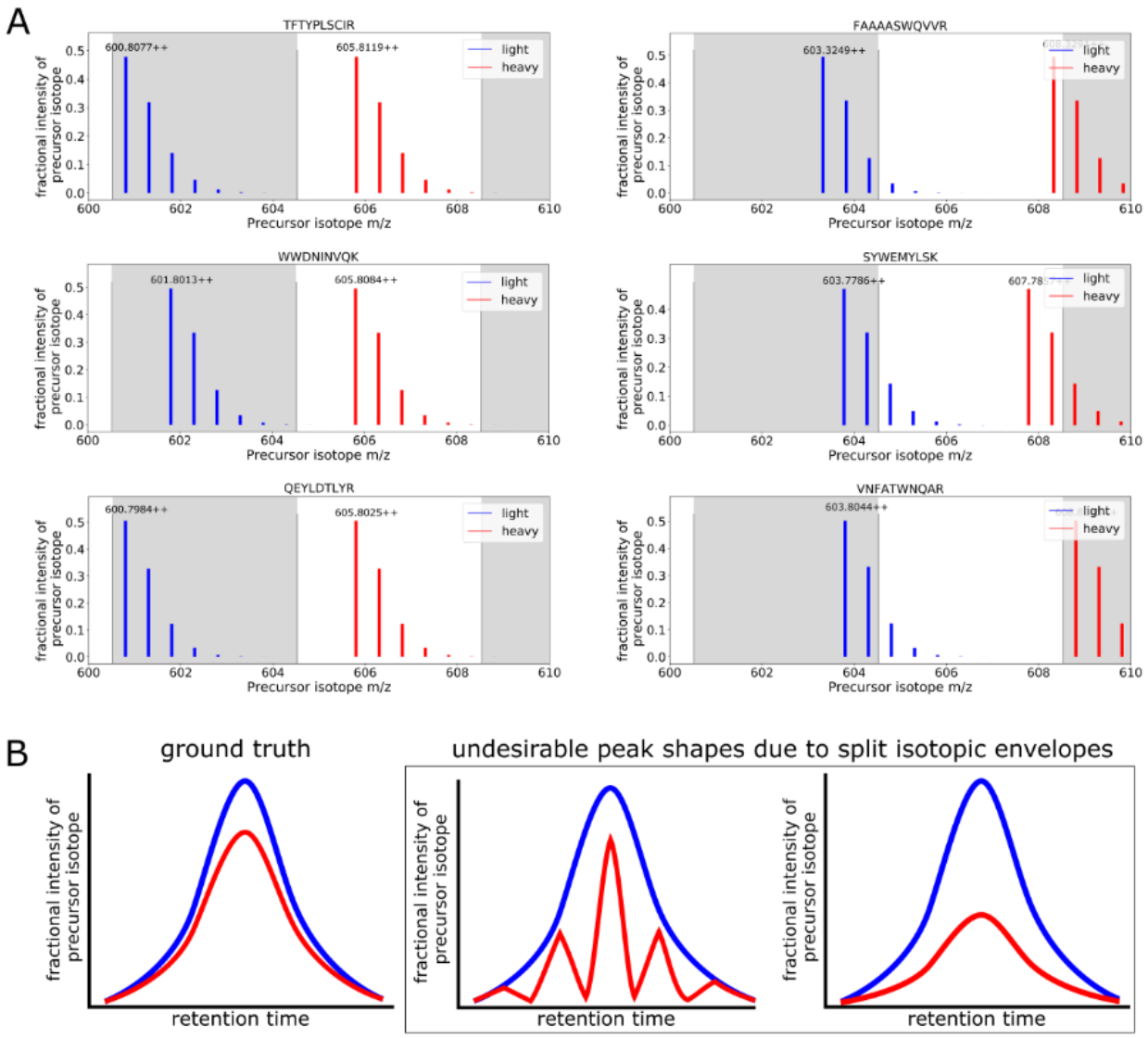
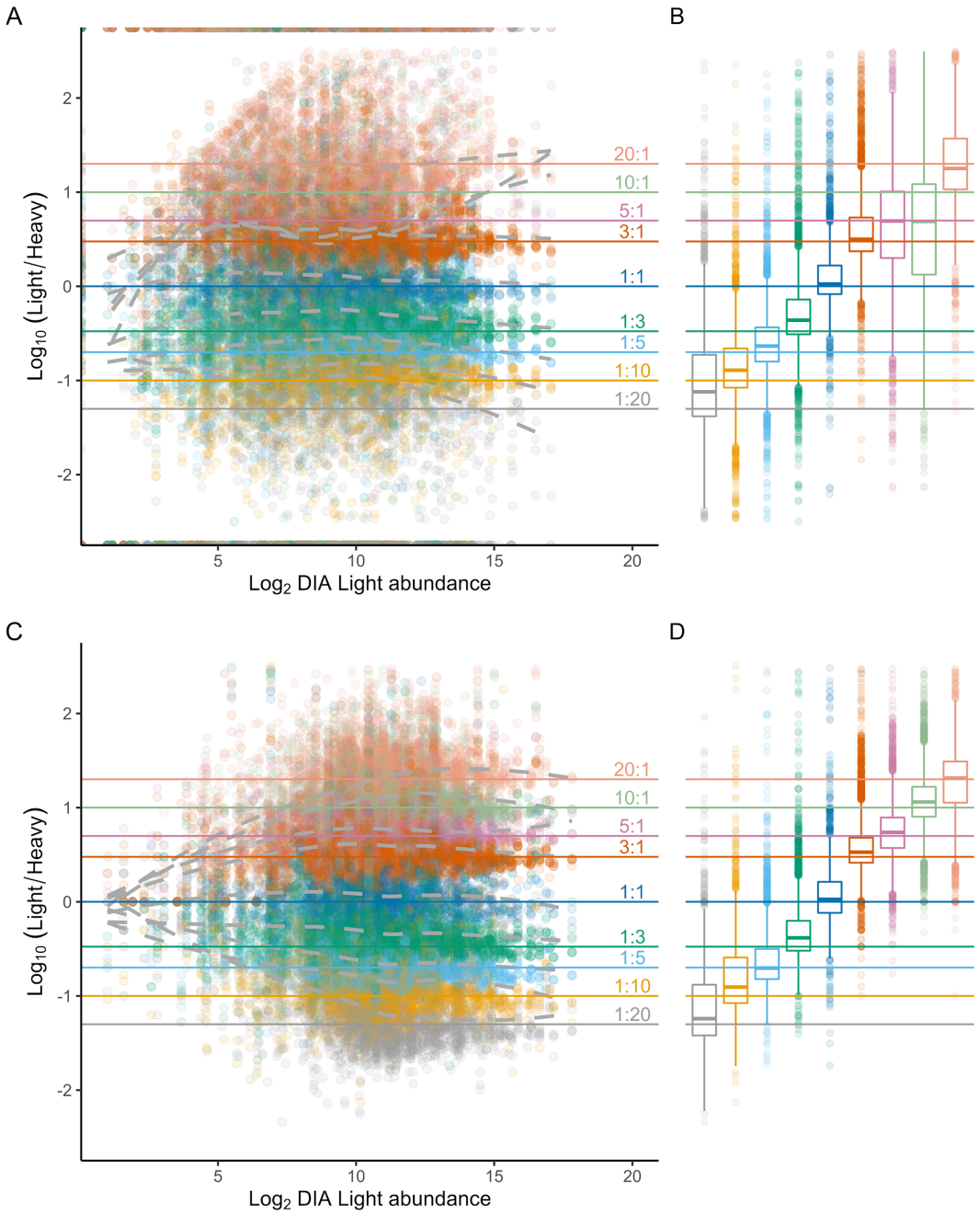
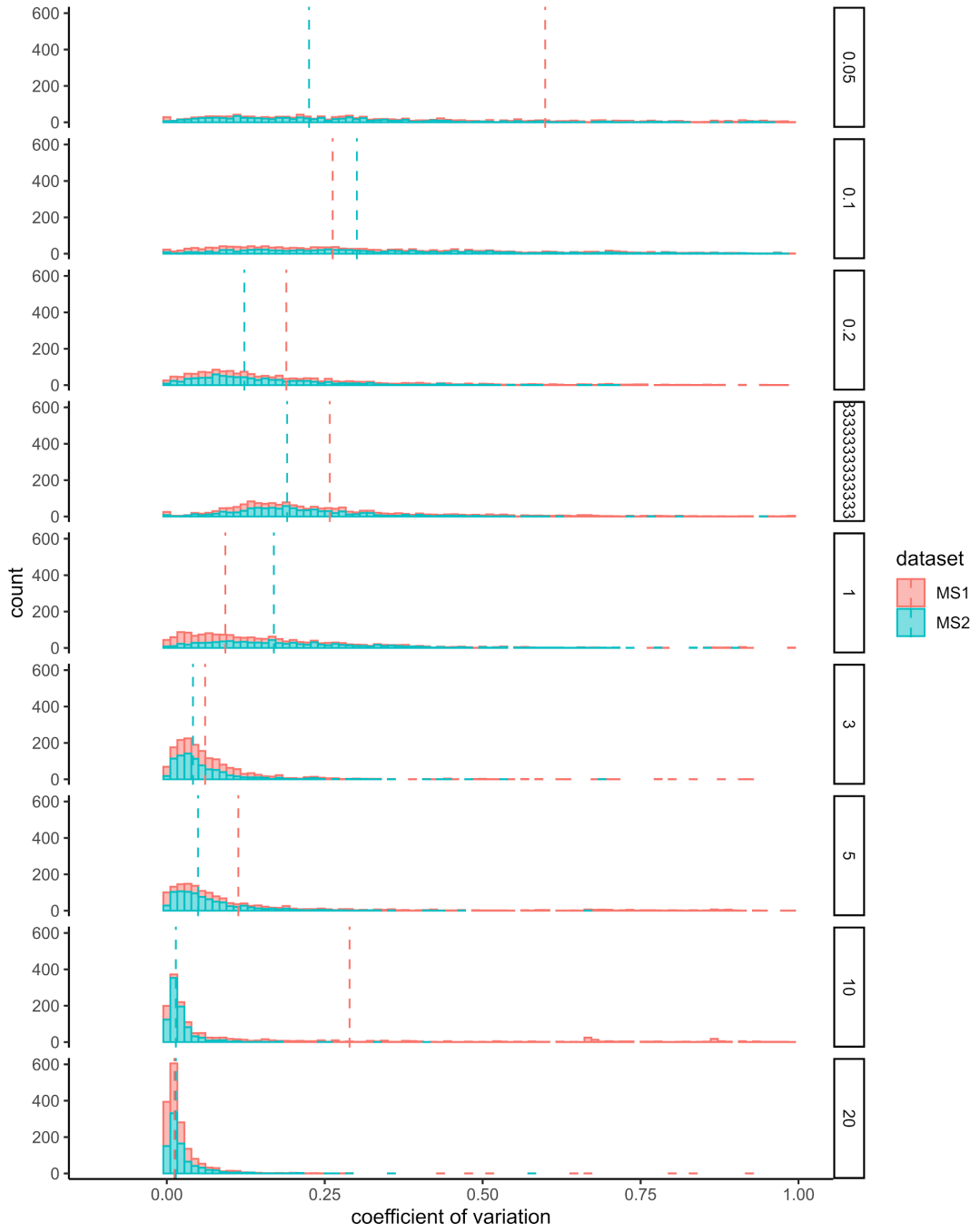


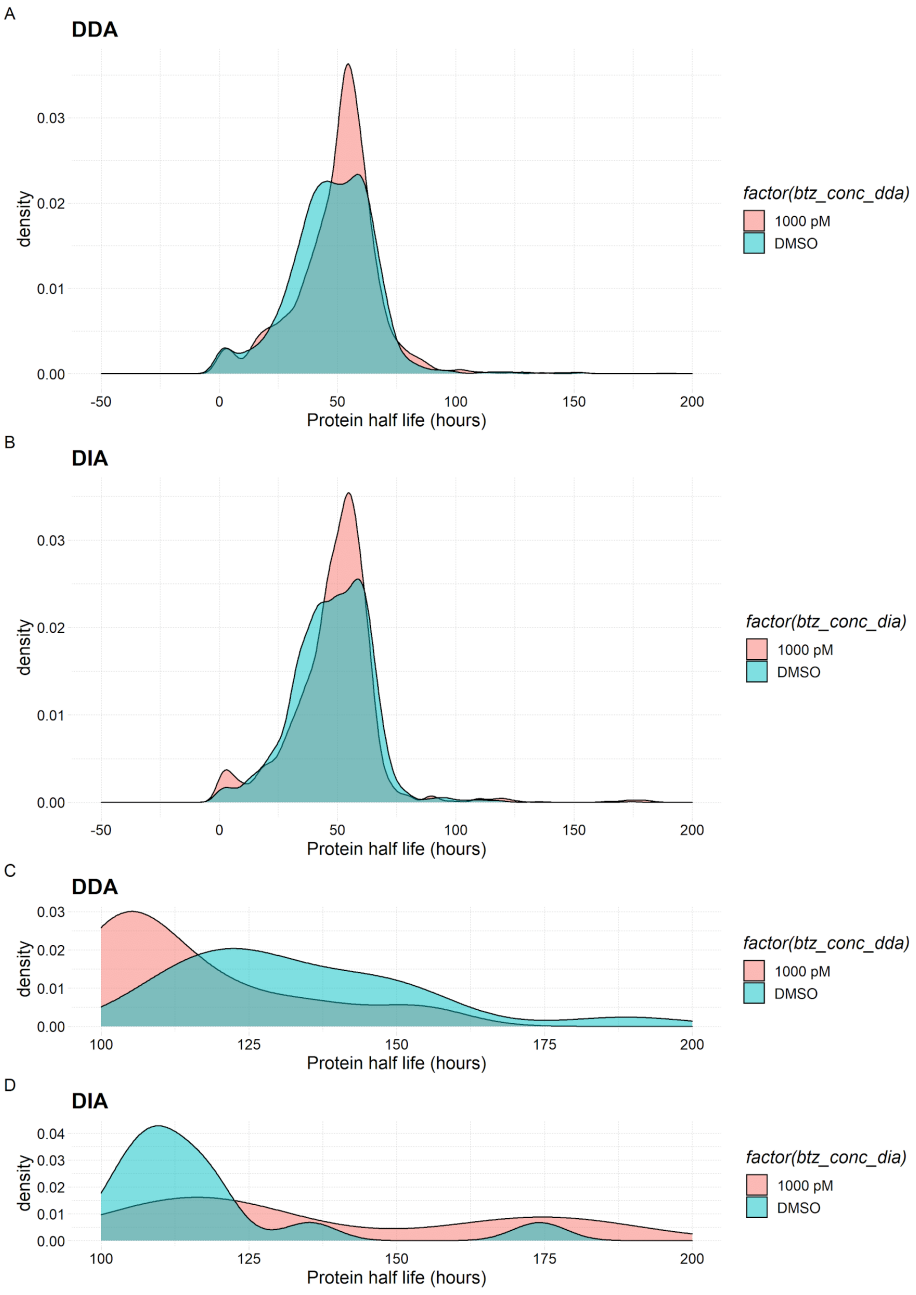
Supplemental Figure 1. Simulation of precursor isotope envelopes of SILAC light and heavy peptides. (A) Six peptides generated from an in silico digestion of the human reference proteome are shown with their light precursor isotopic envelope (blue) and their respective heavy arginine/lysine SILAC isotopic envelope (red). Proposed DIA isolation window boundaries (gray) bound each precursor within a single window (left column), but split the isotopic envelopes of other precursor pairs (right column). **(B)** A ground truth chromatogram for a hypothetical light and heavy precursor pair is shown (left) along with two undesirable chromatograms (right) which may arise due to the heavy precursor having its isotopic envelope split across DIA isolation windows in situations with staggered windows or fixed-width windows.



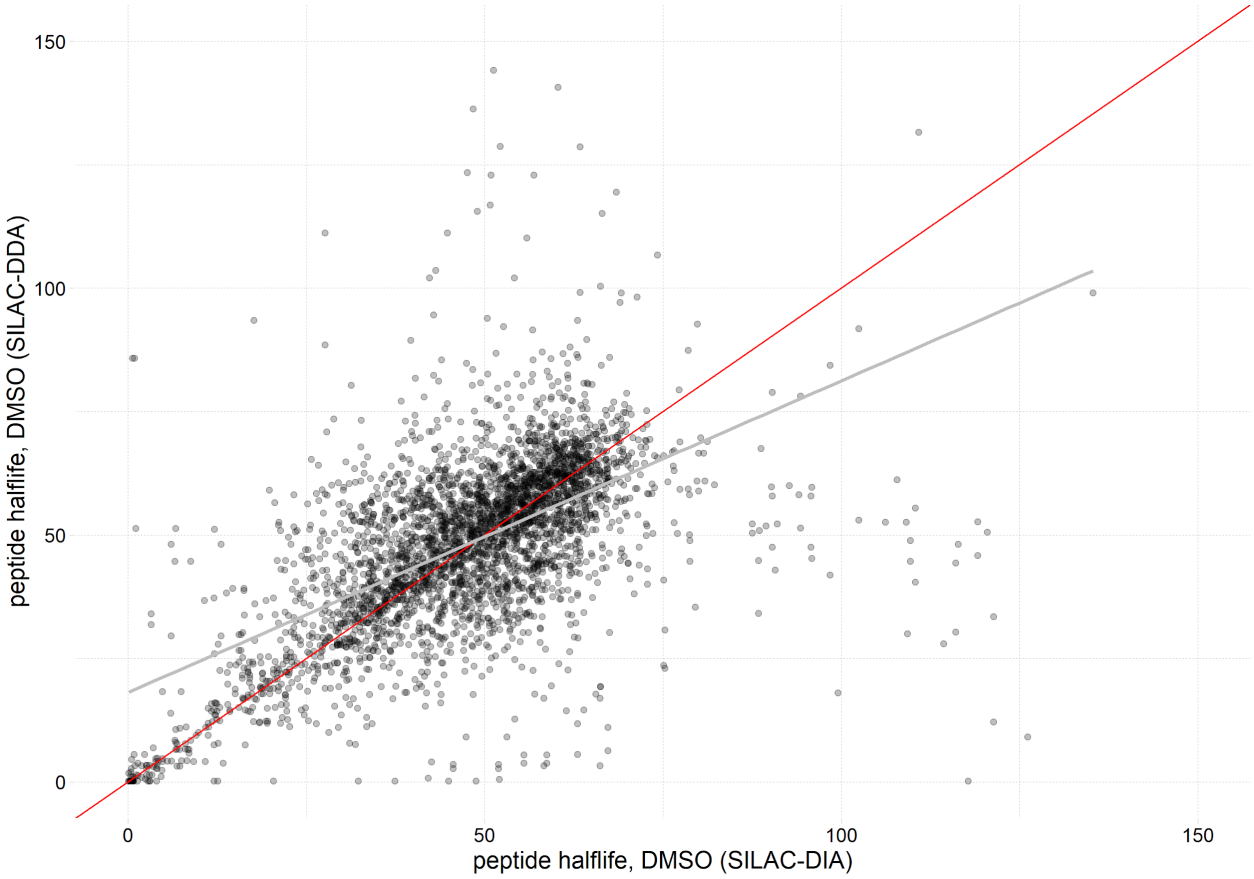
Supplemental Figure 2. SILAC-DIA quantification closely reproduces expected ratios of light/heavy *E.coli* mixtures. The measured log10(light/heavy) ratios in nine dilutions of heavy/light *E.coli* proteome samples are compared using SWATH MS2 quantification. The samples represent dilutions of light *E.coli* into heavy *E.coli* with ratios between 20:1 through 1:20, a 400x range. Data was first searched with EncyclopeDIA, then EncyclopeDIA ELIB results imported into Skyline to perform MS1 precursor and MS2 fragment ion chromatogram extraction, which were used for quantification. The highest rank precursor ion in Skyline was used for MS1 quantification; EncyclopeDIA-refined fragments were used for MS2 quantification.



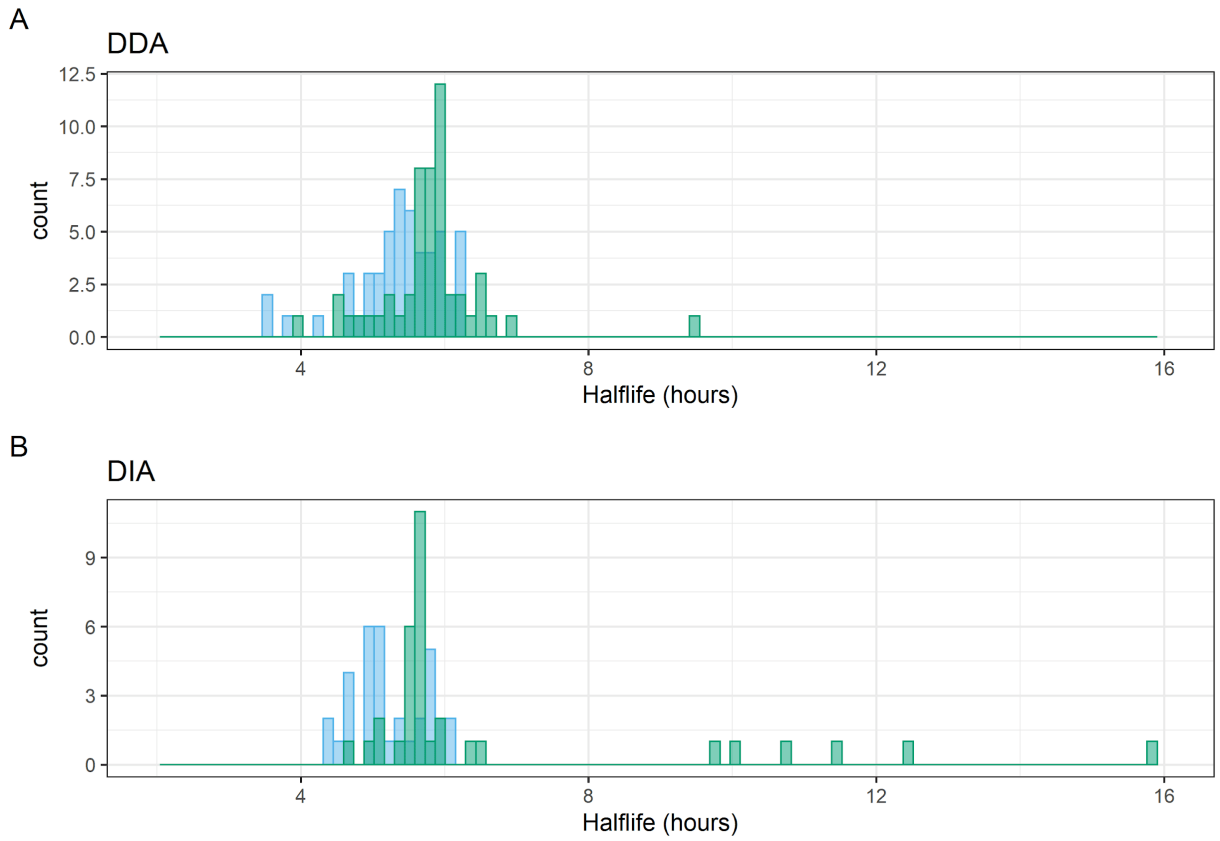
Supplemental Figure 3. MS2 measurements have lower coefficient of variation (CV) than MS1 measurements for SILAC-DIA *E. coli* ratio samples. Heavy and light lysine-labeled *E. coli* samples mixed in nine ratios were quantified using MS1 (top ranked precursor, pink) and MS2 (fragment ion, cyan) data from the same DIA files and the coefficient of variation (CV) calculated across the three technical replicates of each ratio. Dashed lines represent the median CV for that ratio sample and dataset (MS1, MS2). With one exception (ratio sample 1:1), MS2 measurements are comparable or more reproducible (lower CV) than the same peptides quantified by MS1.



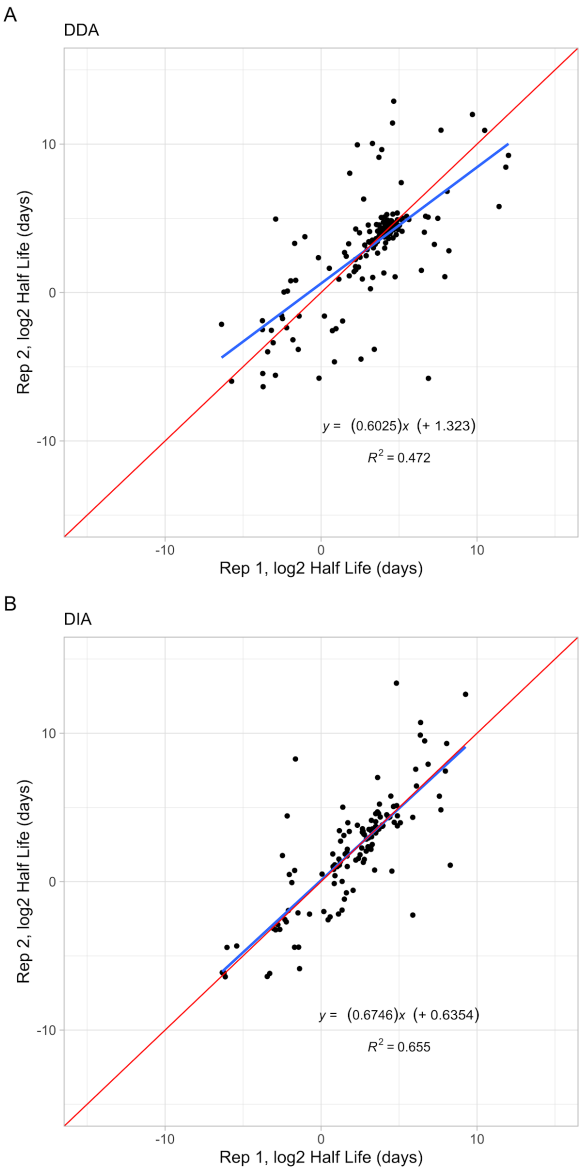
Supplemental Figure 4. Distribution of protein half lives under DMSO and bortezomib, as modeled using DDA and DIA data. Protein half lives based on >2 peptides for each protein are shown as a density distribution for bortezomib treatment (1000 pM, pink) and DMSO control (cyan), with the half lives as calculated by DDA and DIA. **(A,B)** The overall distribution of half life values calculated by DDA and DIA are quite similar. **(C,D)** The more extreme half life values (100-200 hours) are shown to visualize the differences in model sensitivity.



Supplemental Figure 5. Correlation of peptide half lives as calculated by DDA and DIA. Each peptide half life is shown with the value as calculated by DIA against the value as calculated by DDA data. A line of equality (red) is plotted along with a line of best fit (gray). The <1 slope shows that a half life estimated by DDA tends to be smaller (shorter) than a half life estimated by DIA.



Supplemental Figure 6. Distribution of significant protein half lives as assessed by DDA and DIA. The differentially degraded proteins as determined by DDA-based models **(A)** and by DIA-based models **(B)** are shown for bortezomib treatment (green) and DMSO control (blue). Overall, there are more significantly degraded proteins as reported by the DDA-based models, but the DIA-based models include more extreme half life values over 8 hours.



Supplemental Figure 7. Reproducibility of half life estimations between replicates of DDA and DIA pSILAC data. Half lives were calculated for two technical replicates of each DDA and DIA pSILAC data and correlated against each other (blue, line of best fit; red, line of equality with slope of 1 and intercept of 0). **(A)** DDA-based half life estimations between replicates correlated with a slope of 0.6025 and an intercept of 1.323, with an R2 of 0.472. **(B)** DIA-base half life estimations between replicates correlated with a slope of 0.6746 and an intercept of 0.6352, with an R2 of 0.655.

Point	100% Heavy sample (ul)	100% Light sample (ul)	Sample to use for serial dilution	Serial dilution (ul)	Light (ul)	frx dilution Heavy
A	100	0				1
B	70	30				0.7
C	50	50				0.5
D	30	70				0.3
E	10	90				0.1
F			B	10	90	0.07
G			C	10	90	0.05
H			D	10	90	0.03
I			E	10	90	0.01
J			F	10	90	0.007
K			G	10	90	0.005
L			H	10	90	0.003
M			I	10	90	0.001
N	0	100				0

Supplemental Table 1. Dilution scheme for preparing mixture samples of HeLa SILAC light and heavy.

Sample Name	Light E.coli	Heavy E.coli	Percent light	Percent heavy
20:1	20	1	0.95	0.05
10:1	10	1	0.91	0.09
5:1	5	1	0.83	0.17
3:1	3	1	0.75	0.25
1:1	1	1	0.50	0.50
1:3	1	3	0.25	0.75
1:5	1	5	0.17	0.83
1:10	1	10	0.09	0.91
1:20	1	20	0.05	0.95

Supplemental Table 2. Dilution scheme for preparing mixture samples of E.coli SILAC light and heavy.