SUPPORTING INFORMATION

A compendium of murine (phospho)peptides encompassing different isobaric labeling and data acquisition strategies

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Figure S1:

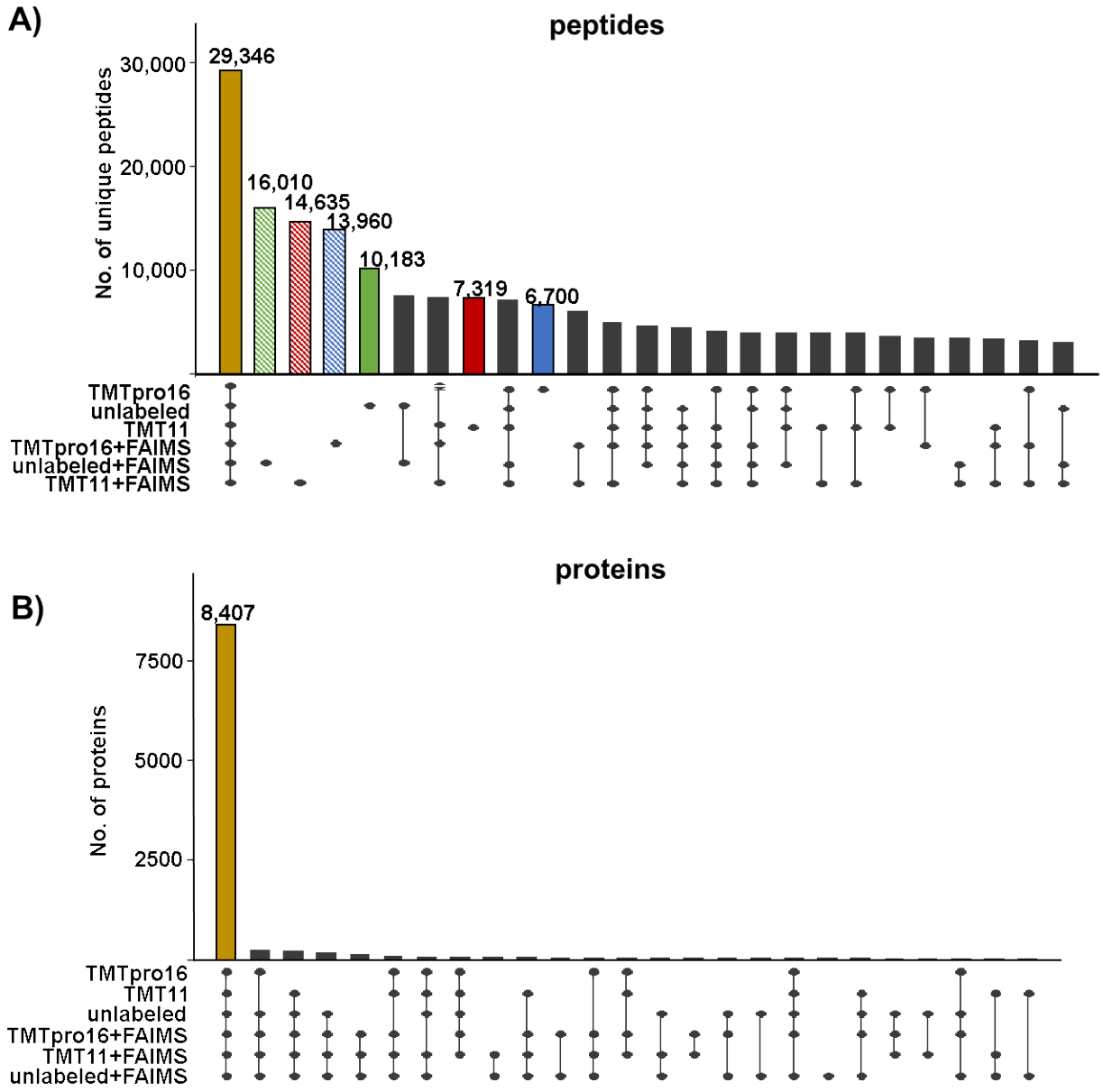

Figure S1: Overlap of the whole proteome tryptic digest datasets across the three labeling strategies. A) Peptide-centric upset plot highlighting the number of peptides identified by a single labeling and acquisition strategy (colored bars). **B)** Protein-centric upset plot highlighting the number of proteins identified in all labeling and acquisition strategy permutations (gold bar). On the upset plots, the bars represent either the number of proteins (panel A) or peptides (panel B) identified. The dots below each bar indicate the experiments in which a given peptide (or protein) has been identified. The twenty-five combinations with the most overlapping proteins are depicted.

Figure S2:

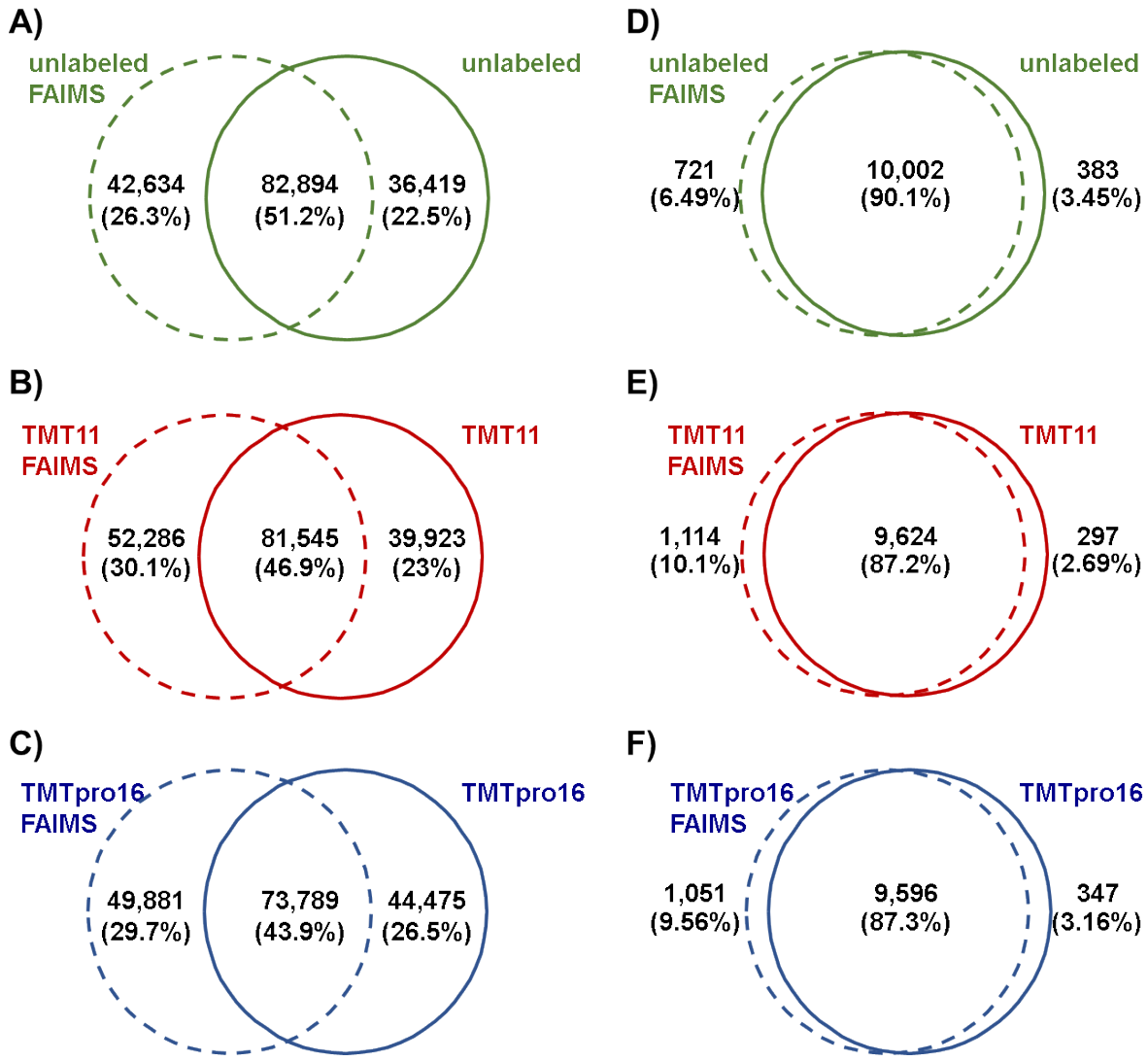

Figure S2: Overlap of differentially labeled peptides from the whole proteome tryptic digest datasets with respect to data acquisition with and without FAIMS-based gas phase separation. Venn diagrams depicting the overlap between peptide-level data acquired with and without FAIMS for the **A)** unlabeled, **B)** TMT11, and **C)** TMTpro16-labeled sample datasets. In addition, Venn diagrams also illustrate the overlap at the protein level of data acquired with and without FAIMS for the **D)** unlabeled, **E)** TMT11, and **F)** TMTpro16-labeled sample datasets.

Figure S3:

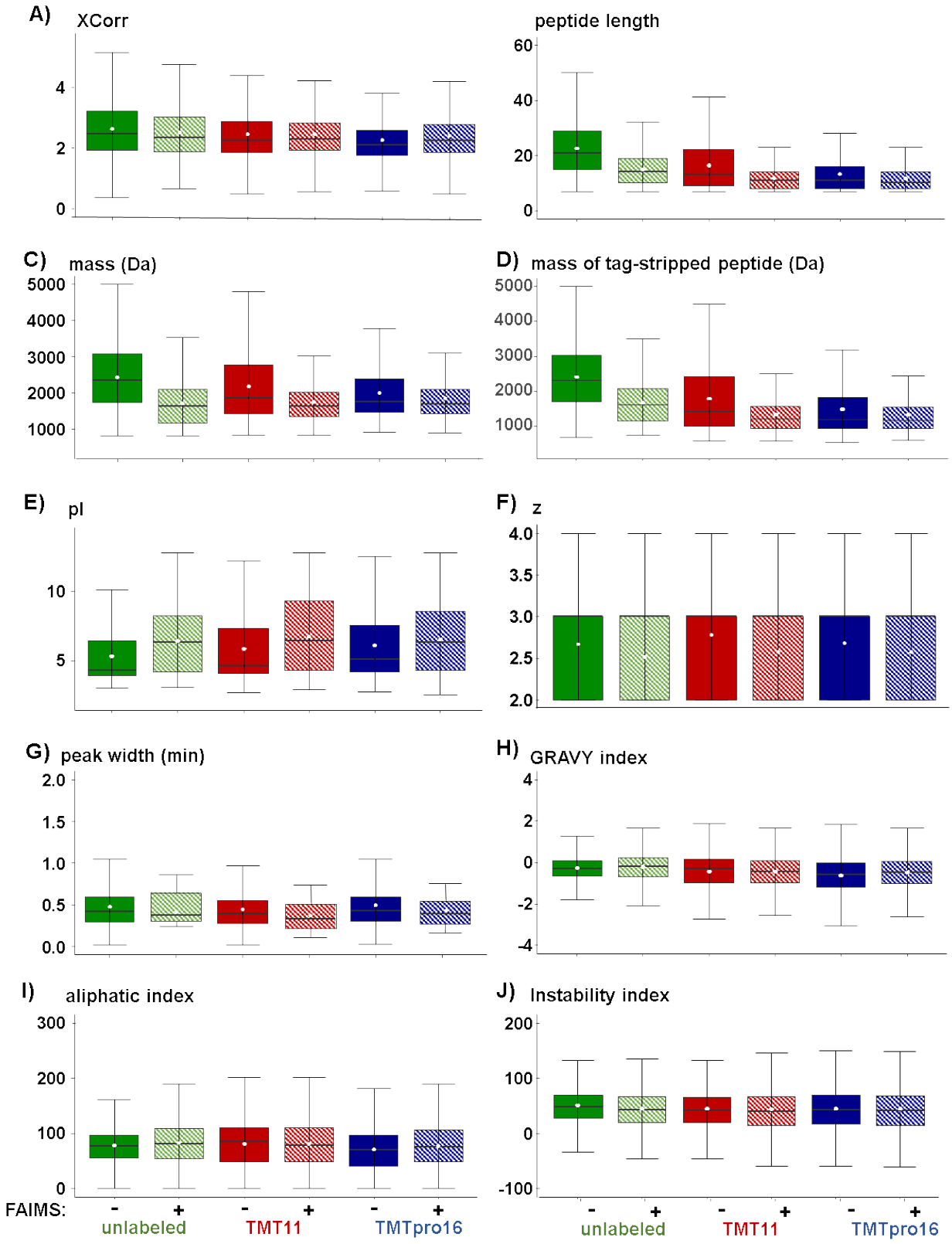
A) XCorr
peptide length
C) mass (Da)
D) mass of tag-stripped peptide (Da)
E) pI
F) z
G) peak width (min)
H) GRAVY index
I) aliphatic index
J) Instability index
FAIMS: - + - + - +
unlabeled
TMT11
TMTpro16

Figure S3: Peptide characteristics. Box-and-whisker plots illustrating the distribution of **A)** XCorr, **B)** peptide length, **C)** mass, **D)** mass of the tag-stripped peptides, **E)** isoelectric point (pI), **F)** z (charge state), **G)** chromatographic peak width, **H)** GRAVY (Grand Average of Hydropathy) index, **I)** aliphatic index, and **J)** instability index. For boxplots, the center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles and the white dot in the box indicates the mean value.

Figure S4:

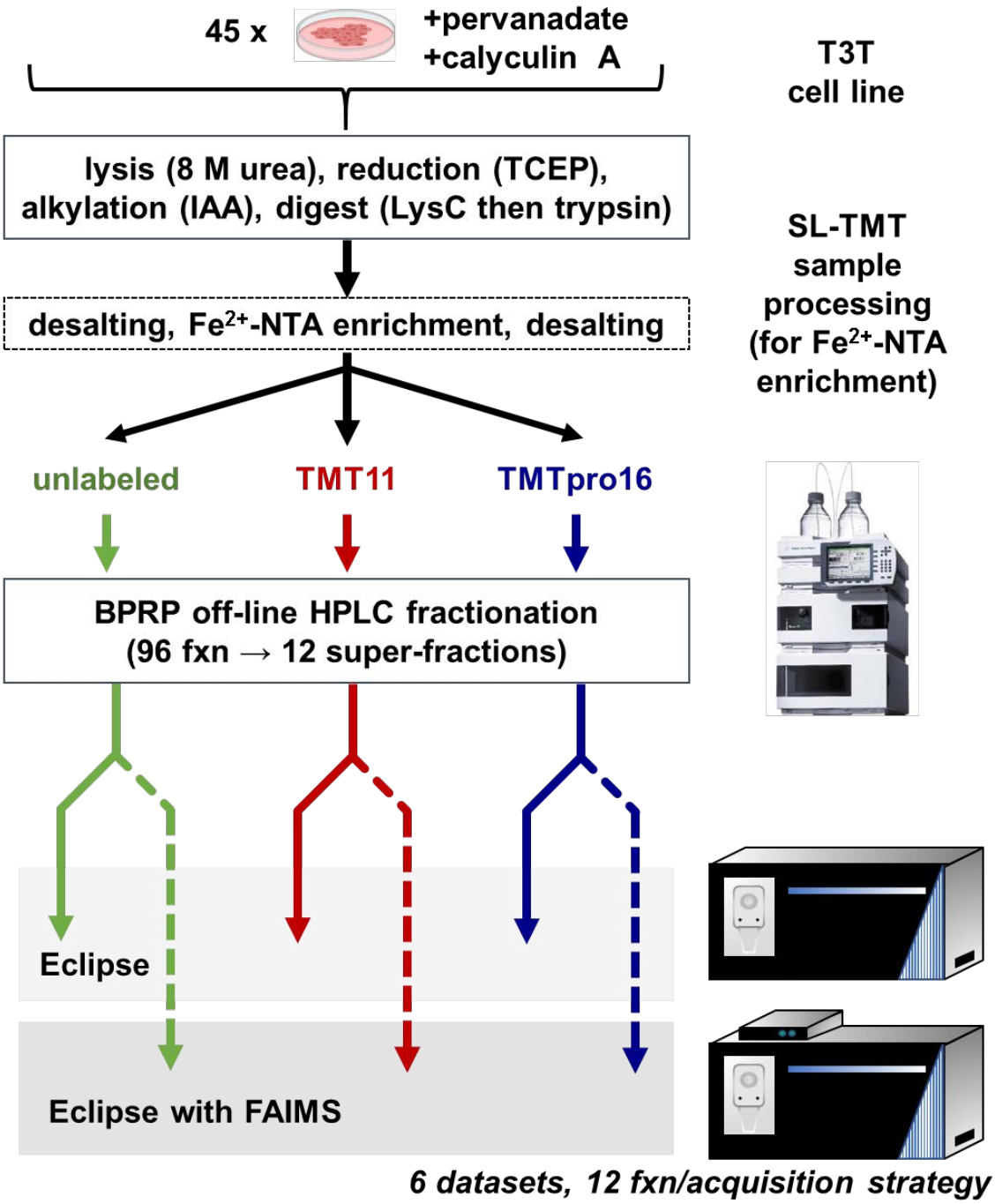

Figure S4: Workflow for Fe^{2+}-NTA-enriched datasets. A mouse embryonic cell line (NIH/3T3) was harvested, lysed, and processed further using the SL-TMT protocol (34). The tryptic peptide pool was enriched for phosphopeptides using Fe^{2+}-NTA and then divided into three aliquots: 1) unlabeled, 2) labeled with TMT11, and 3) labeled with TMTpro16 reagents. These aliquoted peptides were fractionated into 96 fractions using basic-pH reversed-phase (BPRP) chromatography and concatenated into 12 super-fractions. Each super-fraction was analyzed on an Orbitrap Eclipse mass spectrometer with or without FAIMS.

Figure S5:

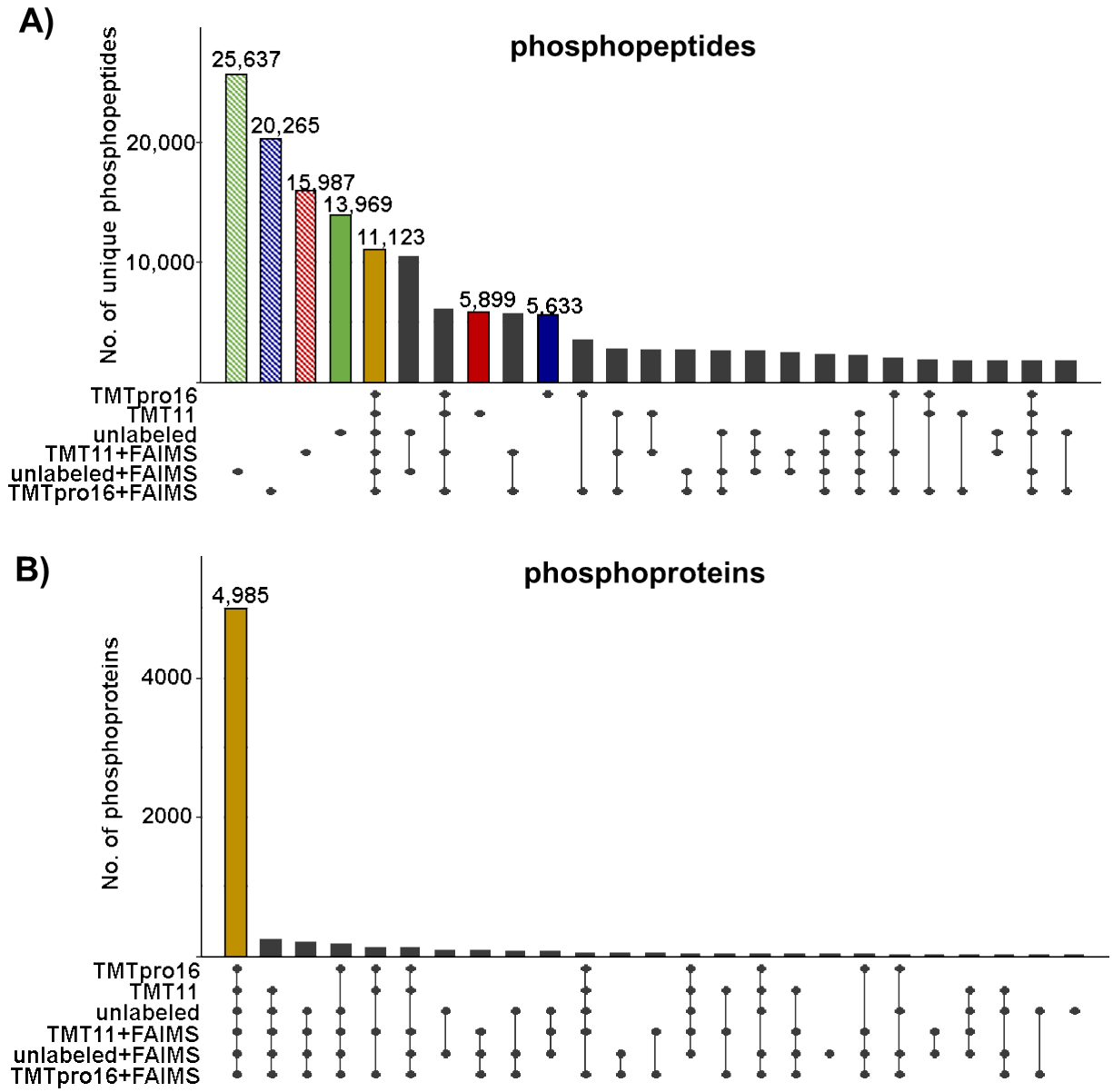

Figure S5: Overlap of the Fe^{2+}-NTA enriched datasets across the three labeling strategies. **A)** Peptide-centric upset plot highlighting the number of phosphopeptides identified by a single labeling and acquisition strategy (colored bars). **B)** Protein-centric upset plot highlighting the number of phosphoproteins identified in all labeling and acquisition strategy permutations (gold bar). On the upset plots, the bars represent either the number of proteins (panel A) or peptides (panel B) identified. The dots below each bar indicate the experiments in which a given peptide (or protein) has been identified. The twenty-five combinations with the most overlapping proteins are depicted.

Figure S6:

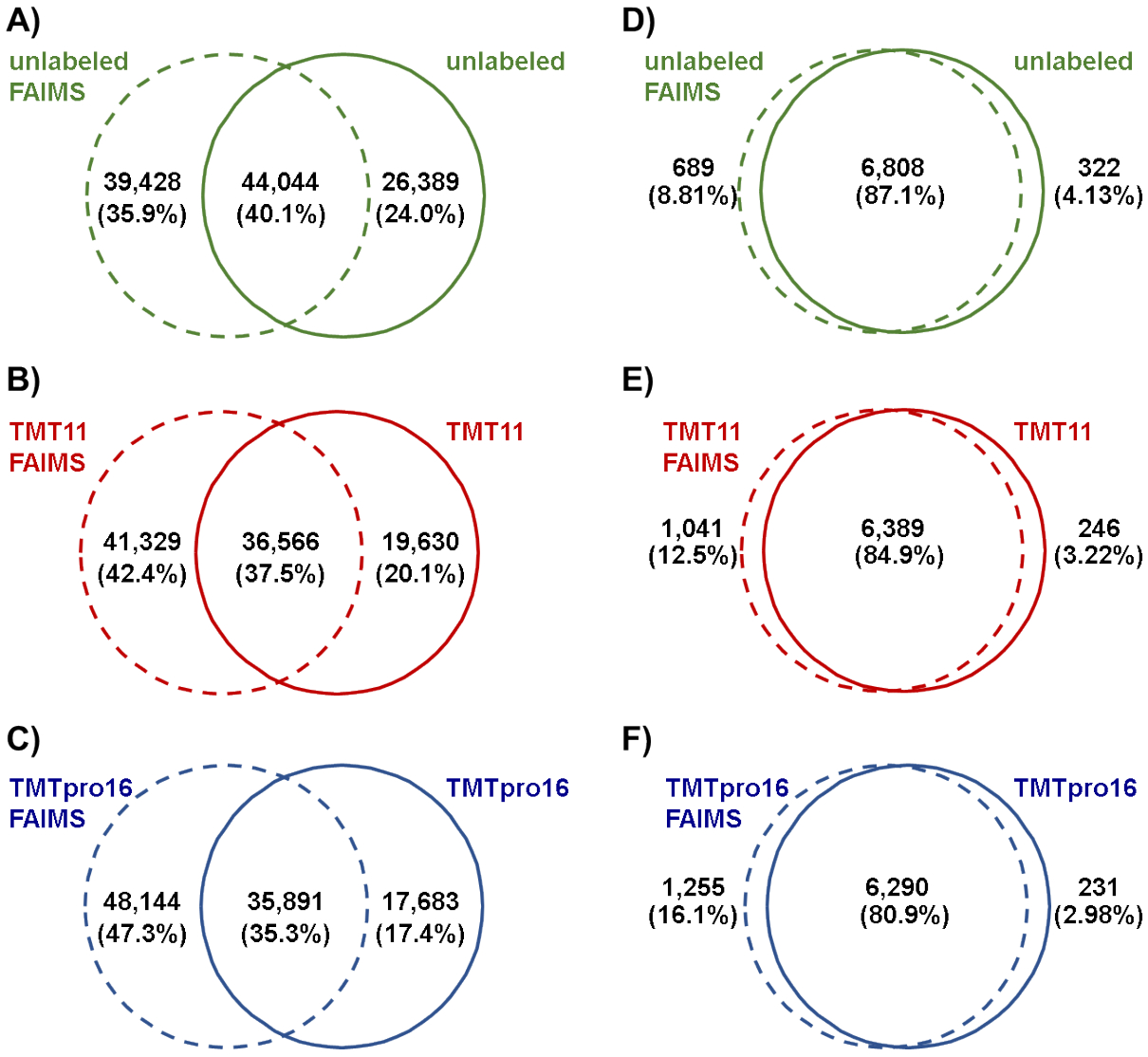

Figure S6: Overlap of differentially labeled phosphopeptides from Fe^{2+}-NTA-enriched datasets with respect to data acquisition with and without FAIMS-based gas phase separation. Venn diagrams depict the overlap between phosphopeptide-level data acquired with and without FAIMS for the **A)** unlabeled, **B)** TMT11, and **C)** TMTpro16-labeled sample datasets. In addition, Venn diagrams also illustrate the overlap at the phosphoprotein level of data acquired with and without FAIMS for the **D)** unlabeled, **E)** TMT11, and **F)** TMTpro16-labeled sample datasets.

Figure S7:

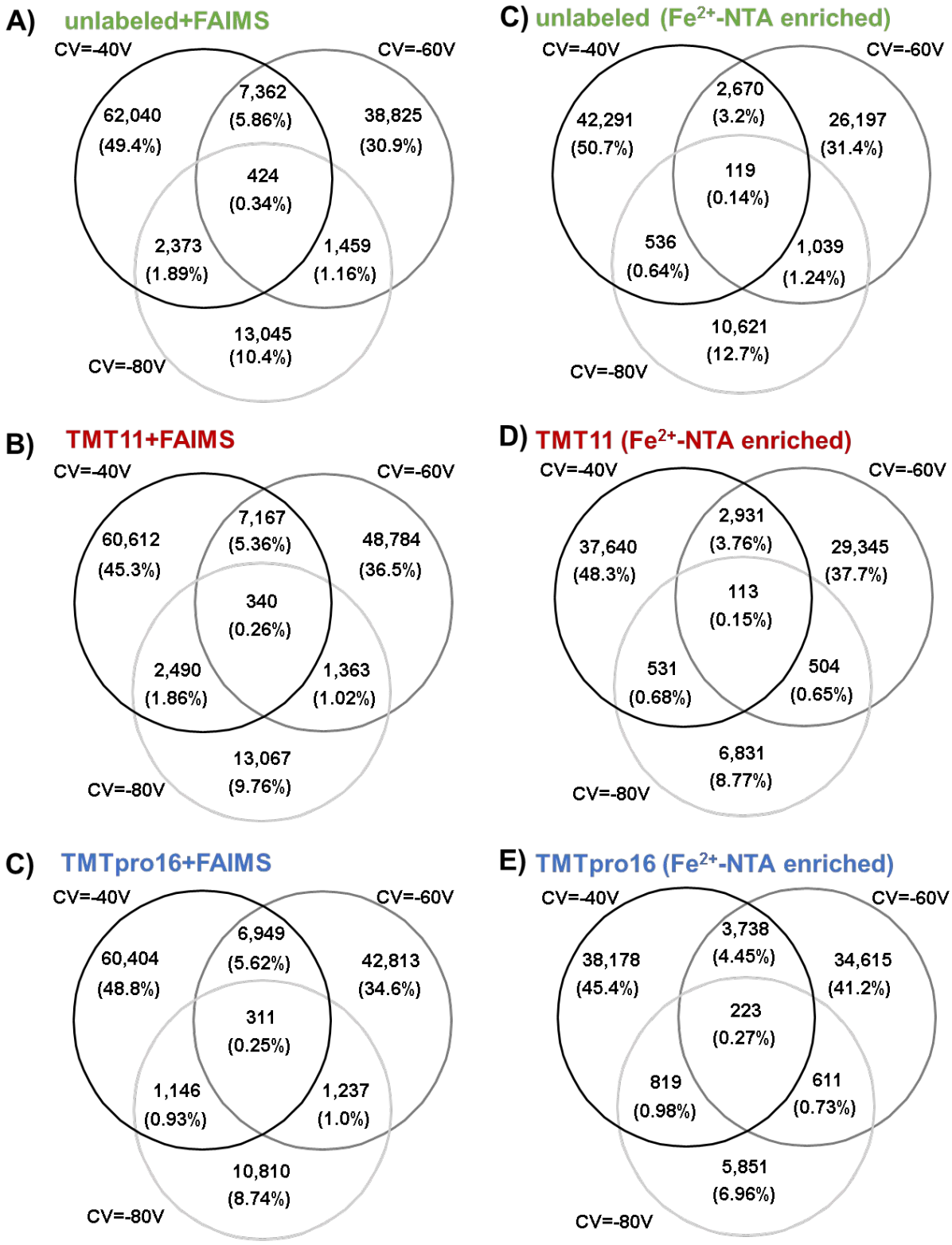
A) unlabeled+FAIMS
CV=-40V
CV=-60V
CV=-80V
62,040
(49.4%)
7,362
(5.86%)
38,825
(30.9%)
424
(0.34%)
2,373
(1.89%)
1,459
(1.16%)
13,045
(10.4%)
C) unlabeled (Fe2+-NTA enriched)
CV=-40V
CV=-60V
CV=-80V
42,291
(50.7%)
2,670
(3.2%)
26,197
(31.4%)
119
(0.14%)
536
(0.64%)
1,039
(1.24%)
10,621
(12.7%)
B) TMT11+FAIMS
CV=-40V
CV=-60V
CV=-80V
60,612
(45.3%)
7,167
(5.36%)
48,784
(36.5%)
340
(0.26%)
2,490
(1.86%)
1,363
(1.02%)
13,067
(9.76%)
D) TMT11 (Fe2+-NTA enriched)
CV=-40V
CV=-60V
CV=-80V
37,640
(48.3%)
2,931
(3.76%)
29,345
(37.7%)
113
(0.15%)
531
(0.68%)
504
(0.65%)
6,831
(8.77%)
C) TMTpro16+FAIMS
CV=-40V
CV=-60V
CV=-80V
60,404
(48.8%)
6,949
(5.62%)
42,813
(34.6%)
311
(0.25%)
1,146
(0.93%)
1,237
(1.0%)
10,810
(8.74%)
E) TMTpro16 (Fe2+-NTA enriched)
CV=-40V
CV=-60V
CV=-80V
38,178
(45.4%)
3,738
(4.45%)
34,615
(41.2%)
223
(0.27%)
819
(0.98%)
611
(0.73%)
5,851
(6.96%)

Figure S7: Overlap of peptides when using different compensation voltages for analysis with FAIMS. Venn diagrams illustrate the overlap of compensation voltages (CV=-80V, -60V, -40V) for the **A)** unlabeled, **B)** TMT11, and **C)** TMTpro16 sample datasets collected with FAIMS for the whole proteome tryptic digest series at the peptide level. Similarly, Venn diagrams depict the overlap of phosphopeptides across compensation voltages (CV=-40V, -60V, -80V) for the **D)** unlabeled, **E)** TMT11, and **F)** TMTpro16 sample datasets collected with FAIMS for the Fe^{2+}-NTA enriched datasets at the peptide level.