

**Supporting Information for**

**METTL21C methylates Alanine tRNA synthetase at lysine 943 in muscle tissue**

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Figure S1 to S5

### **Supplementary Figure Legends**

#### **Supplementary Figure 1. Mass spectrometry analysis of AARS1 as a methylated substrate of METTL21C.**

Representative tandem mass spectra identifying of the *in vitro* methylation of indicated fractions using deuterated SAM as a methyl donor, proteins were in-gel digested by trypsin. Spectra are representative of experiments independently performed three times with similar results. (A-B) MS/MS of the unmodified or monomethylated AARS1 peptide (935aa- 943aa) with the indicated  $m/z$ .  $m/z$  for b and y ions observed in spectra were indicated in blue and red, respectively. (C-E) MS/MS of the mono, di and trimethylated AARS1 peptide (935aa- 962aa) with the indicated  $m/z$ . Digested peptides analyzed by Fusion Mass spectrometer.

#### **Supplementary Figure 2. Mass spectrometry analysis of methylated recombinant AARS1.**

Representative tandem mass spectra of (A) unmodified, (B) mono and (C) di-methylated GST-AARS1 (935aa- 943aa) peptide after *in vitro* methylation of AARS1 by METTL21C using deuterated SAM as a methyl donor. Proteins were in-gel digested by GluC.  $m/z$  for b and y ions observed in spectra are indicated in blue and red, respectively.

#### **Supplementary Figure 3. Sequence alignment of METTL21 family.**

Protein sequence alignment of the METTL21 family of enzymes. METTL21A, B, C and D sequences alignments were performed with the MUSCLE algorithm using Jalview software. Conserved catalytic residues between the METTL21 family members is indicated with arrows. The colors in the figure indicating the default color scheme used for alignments in ClustalX [38, 39]. Blue- Hydrophobic, red- positive charged, magenta- negative charge, green- polar, pink- cystines, orange- glycines, yellow- prolines, cyan- aromatic.

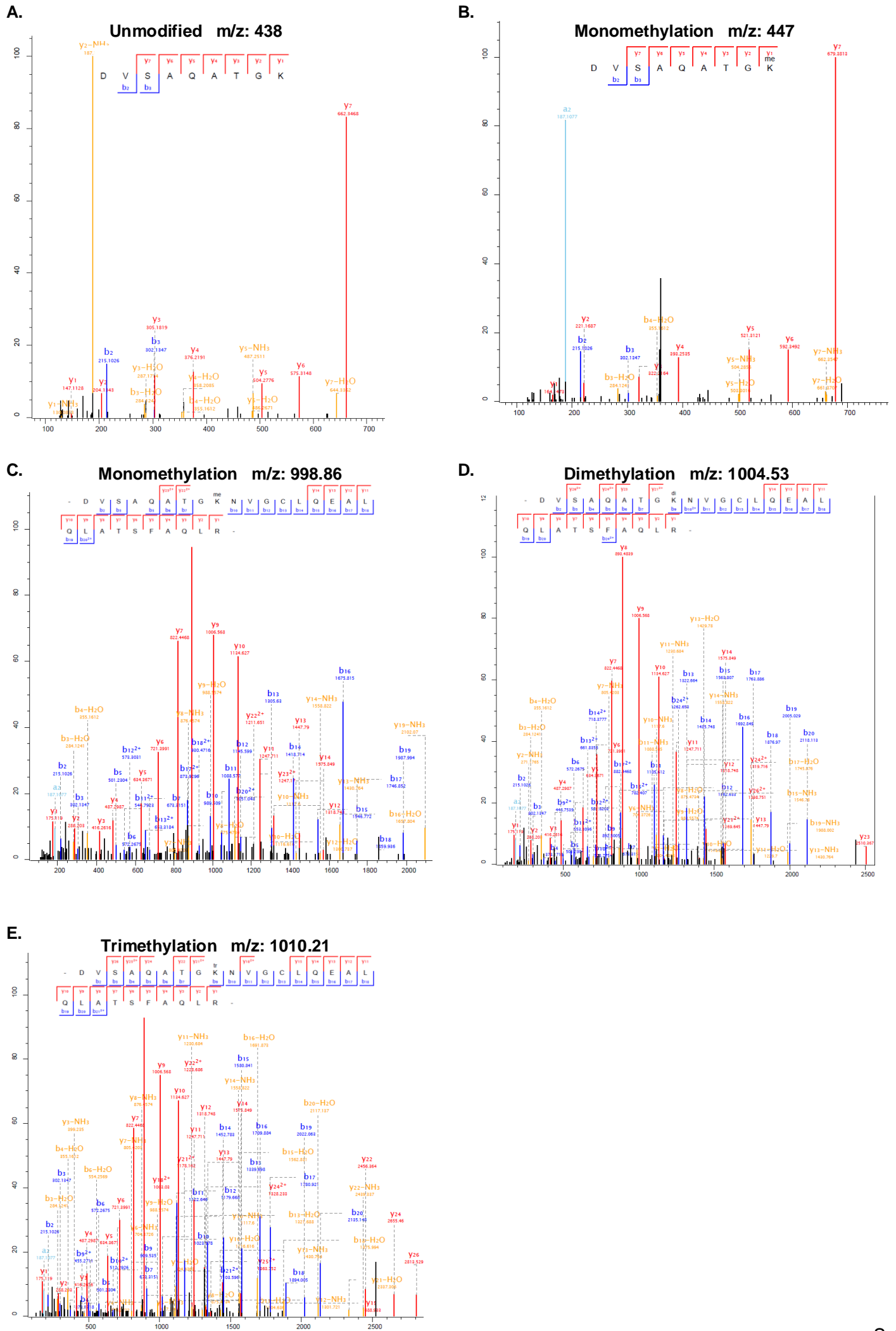
#### **Supplementary Figure 4. Mass spectrometry analysis of GFP-AARS1 methylation in METTL21C expressing cells.**

Representative LC-MS/MS analysis of GFP-AARS1 after IP from 293T transfected cells, proteins were in-gel digested by Glu-C. (A-D) Representative tandem mass spectra analysis of the (A) unmodified, (B) mono-methylated (C) di-methylated (D) tri-methylated peptide of AARS1 with the indicated  $m/z$ .  $m/z$  for b and y ions observed in spectra were indicated in blue and red, respectively. Digested peptides analyzed by Elite Mass spectrometer.

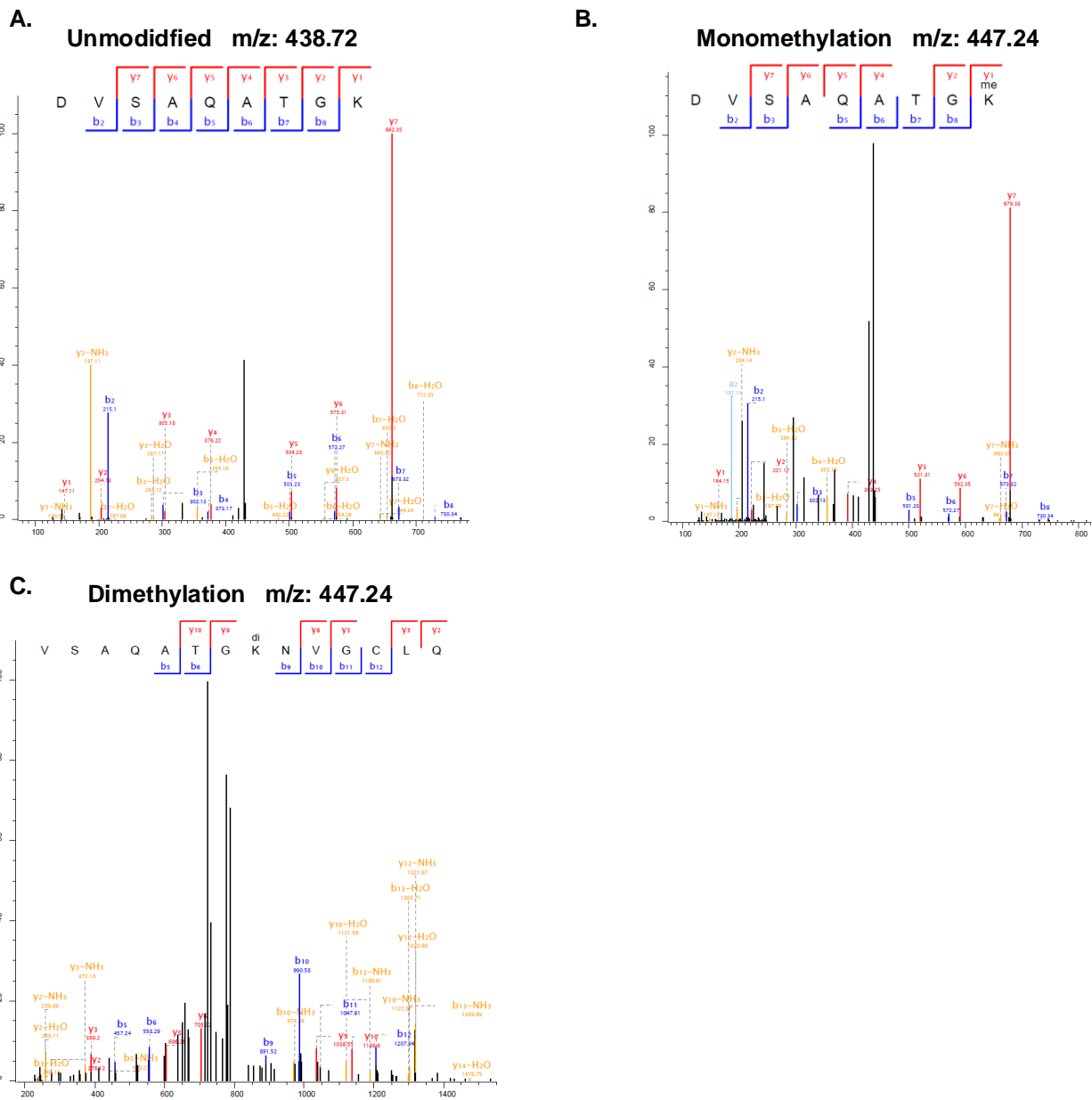
#### **Supplementary Figure 5: Mass spectrometry analysis of AARS1 methylation in METTL21C expressing cells.**

Representative tandem mass spectra analysis of AARS1 after IP from 293T cells using anti-AARS1 antibody, proteins were in-gel digested by Glu-C. (A-D) Representative tandem mass spectra analysis of the (A) unmodified, (B) monomethylated (C) demethylated (D) trimethylated peptide of AARS1.  $m/z$  for b and y ions observed in spectra were indicated in blue and red, respectively. Digested peptides analyzed by Elite Mass spectrometer. (E) i- Chromatograms for quantification of GFP-AARS1 from Flag-METTL21C transfected cells (see Supplementary Figure 4), ii- table represents the peptide quantification. (F) i- Chromatograms for quantification of AARS1 from Flag-METTL21C transfected cells, ii- table represents the peptide quantification.

# Zoabi et al., Supplementary Figure 1

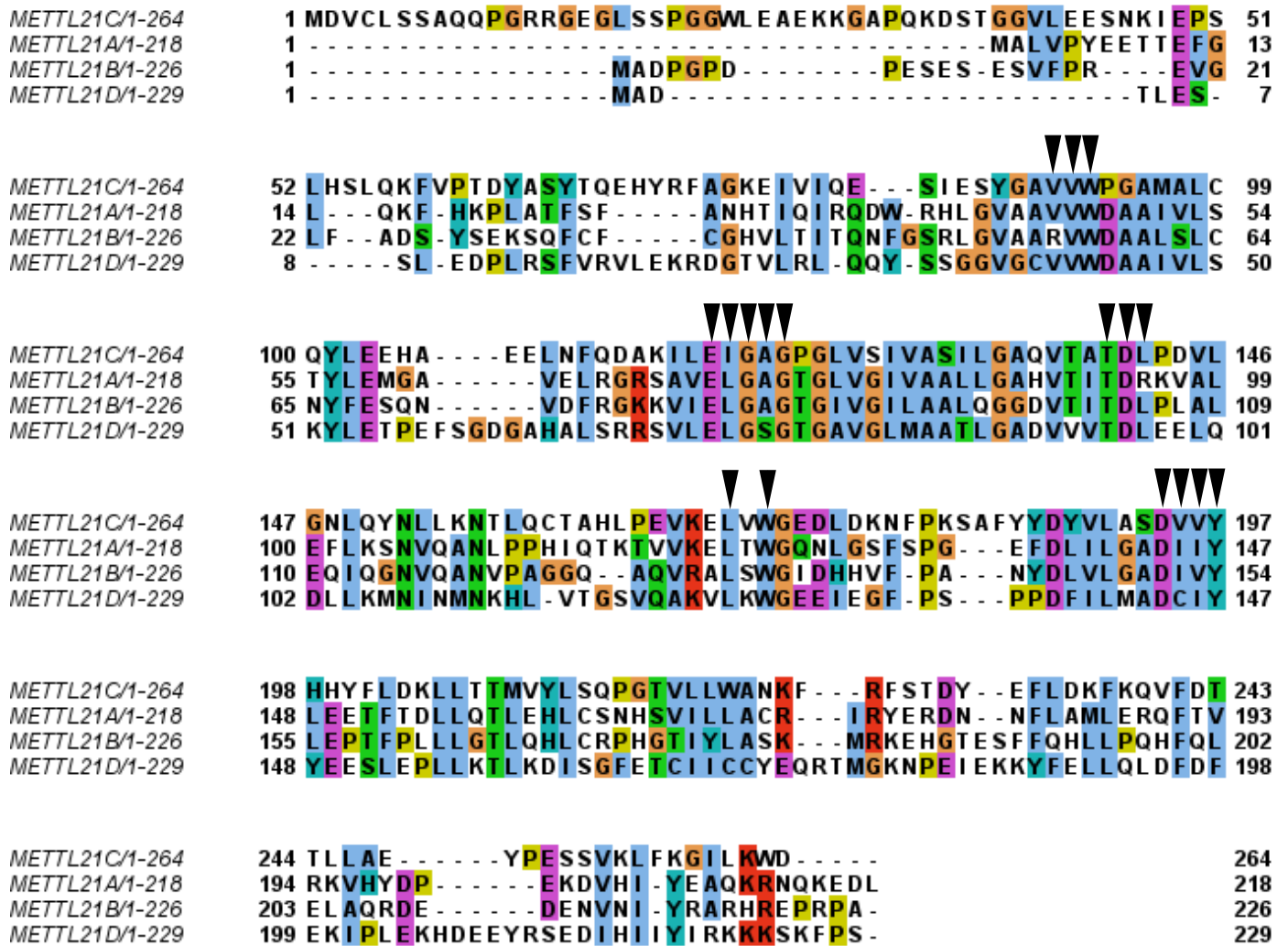


# Zoabi *et al.*, Supplementary Figure 2



# Zoabi et al., Supplementary Figure 3

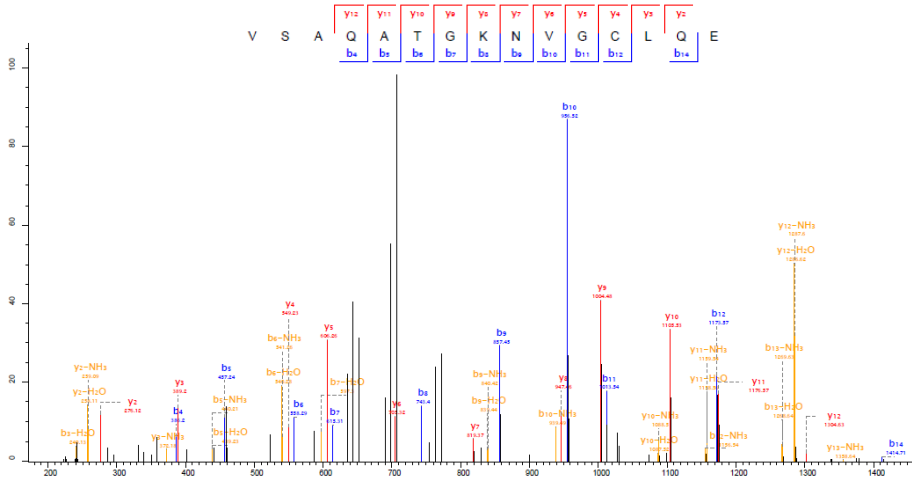
A.



# Zoabi *et al.*, Supplementary Figure 4

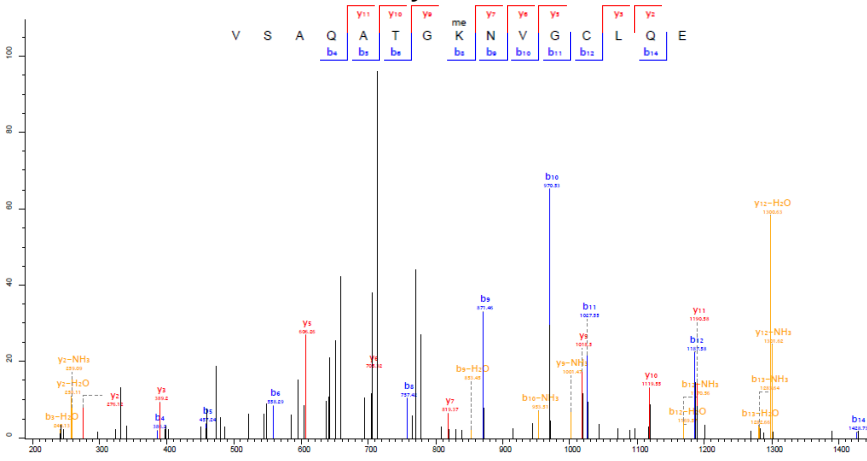
A.

## Unmodified m/z: 781.39



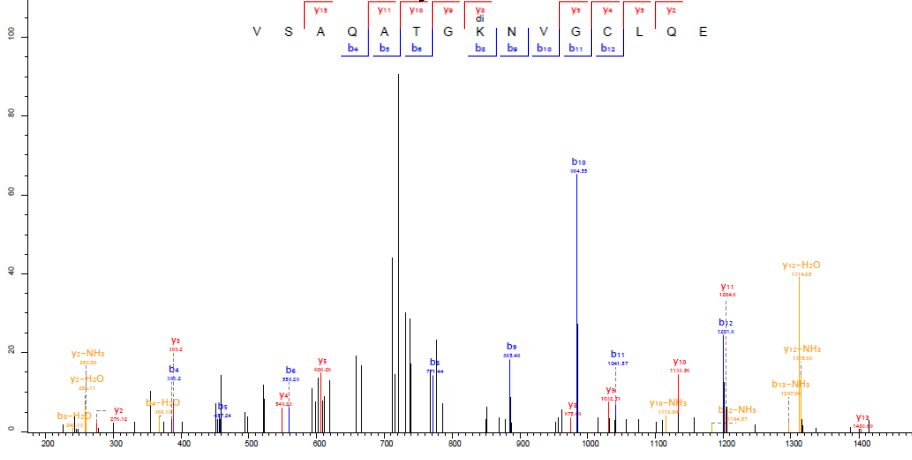
B.

## Monomethylation m/z: 788.39



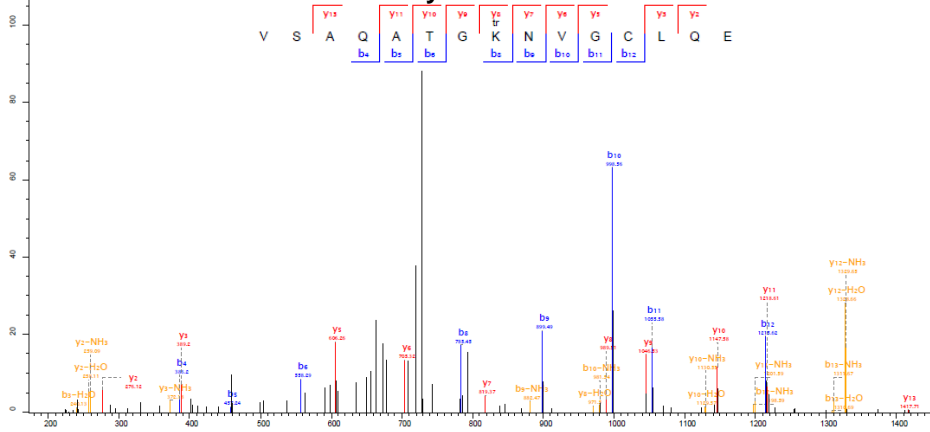
C.

## Dimethylation m/z: 795.4



D.

## Trimethylation m/z: 802.41



# Zoabi et al., Supplementary Figure 5

