

## Supplementary Document: Targeted analysis of biomarker candidates using DIA and Skyline

**Figure 1.** Quantification of the quality control (QC) peptide K.TASEFDSAIAQDK.S from the spiked Thermo RT standard (5fmol; Supplementary methods) in Skyline. **(a)** MS1 (upper) and corresponding MS2 (lower) peak area for this peptide in the 106 DIA samples run, including 38 PE cases and 66 healthy controls. The isotopic distribution “idotp” is excellent, ranging between 0.97 and 1 for MS1, and between 0.75 and 0.96 for MS2. The samples are ordered along the x-axis according to the time at which they were acquired by the mass spectrometer. It is important to note the peak areas displayed have not been normalised; for subsequent analysis in mapDIA the peak area data was first log transformed, and then normalised using the total ion sum, prior to statistical analysis between groups. **(b)** Chromatographic peak view of the QC K.TASEFDSAIAQDK.S peptide in Skyline which illustrates the MS1 precursor peak area (upper) and corresponding MS2 fragments (lower) in a control sample. The intensity of the peak ( $10^6$ ) is represented on the y-axis with retention time (minutes) shown on the x-axis. Peak boundaries are marked by dotted black lines on the y-axis, and the blue lines indicate a confident match with the Skyline library. Mass accuracy for MS1 is -0.8ppm and -0.5ppm for the corresponding MS2 peak. **(c)** Total peak area for the K.TASEFDSAIAQDK.S peptide at MS1 (upper) and corresponding MS2 levels (lower) grouped for Controls (n=66) and PE (n=38). The coloured lines within each block correspond to the contribution to the total peak area from any given precursor (MS1) or fragment (MS2) ion **(d)** Summed Peak area for all fragments of K.TASEFDSAIAQDK.S (MS1 and MS2) in both conditions, Controls (n=66) versus PE (n=38). **(e)** The Coefficient of Variation (CV) for the corresponding peak is represented on the y-axis as a decimal value, whereby a peak area CV  $\leq$  0.4 for Controls (n=66) and peak area CV  $\leq$  0.8 for PE (n=38) was observed (x-axis).

**Figure 2.** Targeted quantification of biomarker candidate **C4BPA** in the PE cohort (n=106) using DIA and Skyline. **(a)** Seven peptides from the C4BPA protein sequence were deemed suitable for quantification in Skyline, highlighted in red in the protein sequence above. **(b)** These peptides were eluted over a 90min gradient (Retention Time; x-axis) and varied in intensity ( $10^9$ ; y-axis) throughout the DIA run. **(c)** Targeted quantification of these seven peptides in controls (n=66) and PE cases (n=38) confirmed a significant decrease in C4BPA in PE cases.

**Figure 3 (a)** Total peak area of the seven **C4BPA** peptides targeted in the PE cohort using DIA and Skyline. Peak area ( $10^9$ ) is represented on the y-axis for both the controls (n=66) and PE cases (n=38), on the y-axis. **(b)** The corresponding peak area - Coefficient of Variation (CV) is represented on the y-axis, whereby the CV is  $<0.1$  for all peptides quantified for both the controls (n=66) and PE cases (n=38).

**Note:** Skyline software images (figures 1, 3a and 3b) have been modified in Powerpoint for legibility i.e. axes labels and scale have been increased in size.

**Figure 1.** Quantification of the quality control (QC) peptide K.TASEFDSAIAQDK<sub>S</sub>

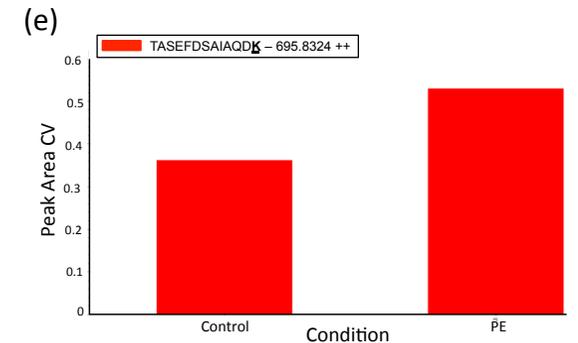
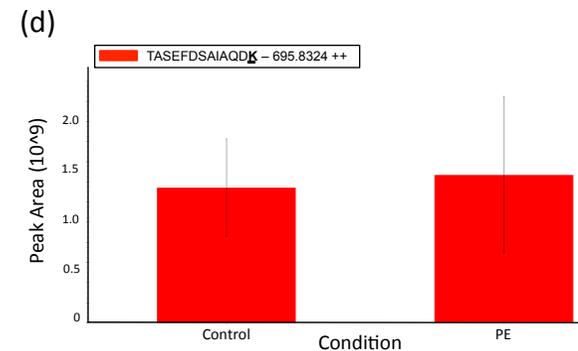
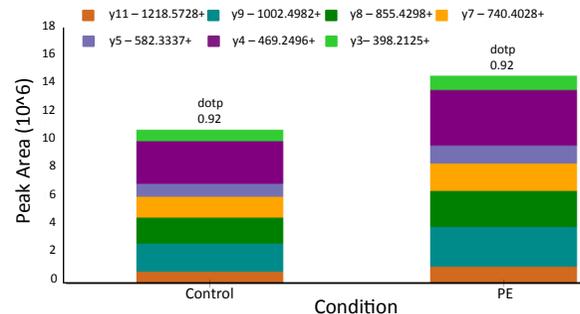
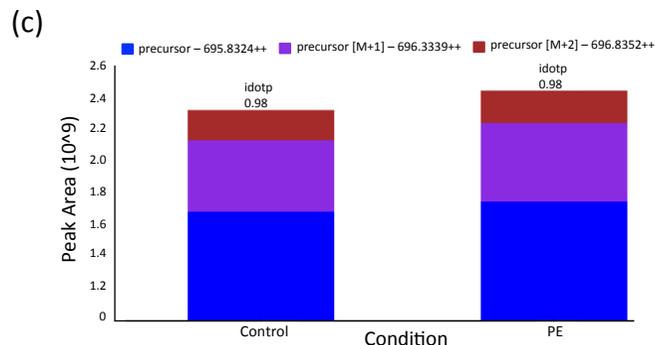
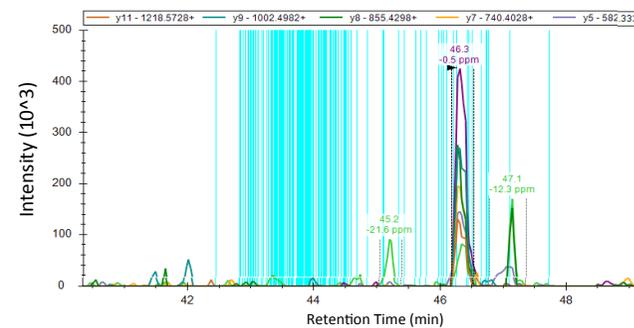
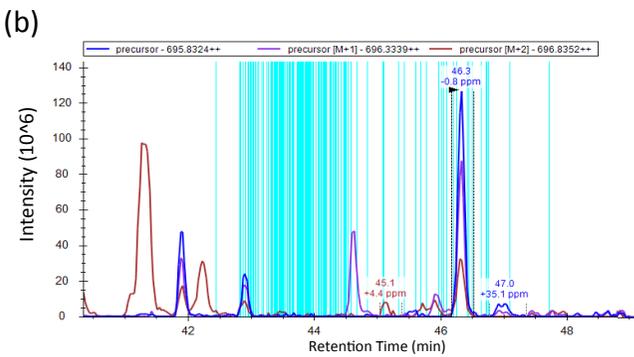
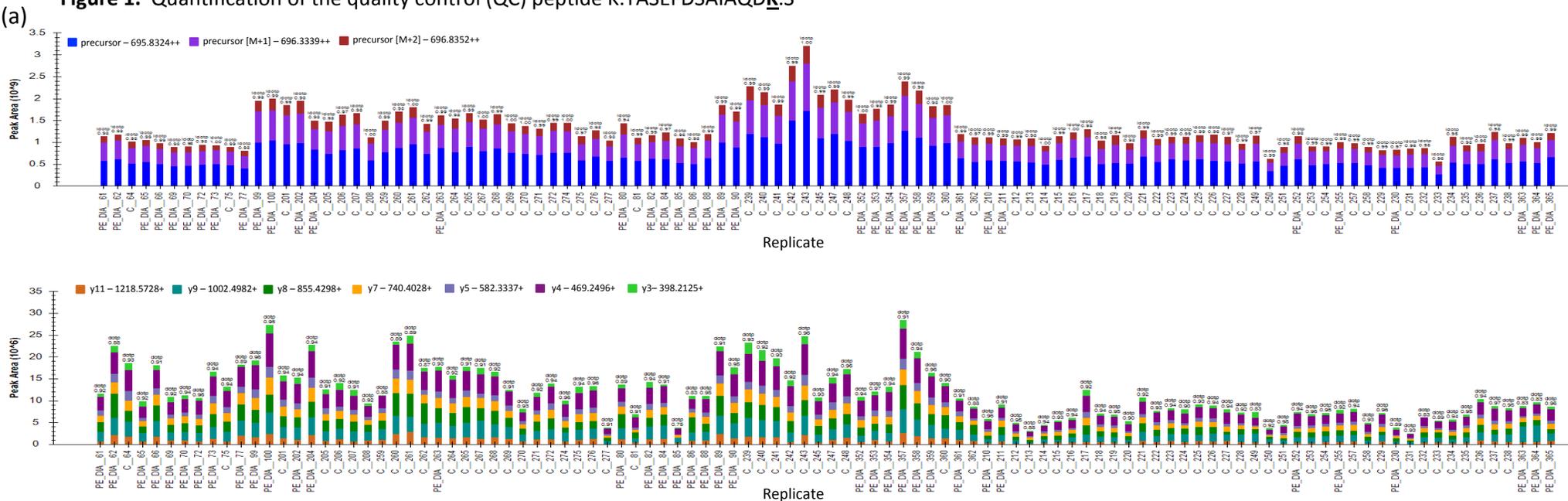


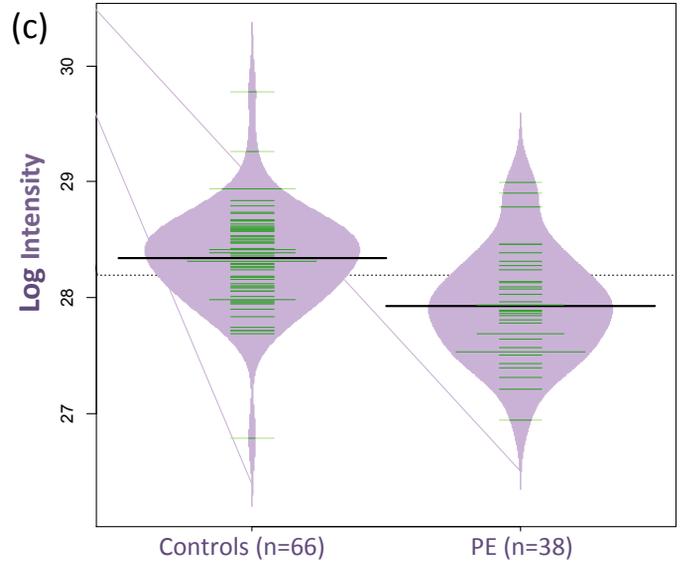
Figure 2. (a) | P04003 | C4BPA\_HUMAN C4b-binding protein alpha

**SEQUENCE:**

MHPPKTPSGALHRKRKMAAWPFSRLWKVSDPILFQMTLIAALLPAVLGNCGPPPTLSFAAPMDITLTETRFKGTTLK  
**YTCLPGYVR**SHSTQTLTCNSDGEWVYNTFCIYKRCRHPGELRNGQVEIKTDLSEFGSQIEFSCSEGFLLIGSTTSRCEVQD  
**RGVGWSHPLPQCEIVK**CKPPDIRNGRHSGEENFYAYGFSVTYSCDPRFSLGHAISICTVENETIGVWRPSPPTCEKI  
 TCRKPDVSHGEMVSGFGPIYNYKDTIVFKCQKGFVLR**GSSVIHCADADSK**WNPSPPACEPNSCINLPDIPHASWETYP  
 RPTK**EDVYVVGTVLR**YRCHPGYKPTTDEPTTVICQKNLRWTPYQGCEALCCPEPKLNNGEITQHRKSRPANHCVYFY  
 GDEISFSCHETS**RFAICQGDGTWSPRTPSCGDICNFPPKIAHGHYKQSSSYFFKEEIIYCDK**GYILVGQAKLSCSYS  
**HWSAPAPQCKALCRKPELVNGLSVDKDKQYVEPVNTIQCDSGYGVVGPQSITCSGNRTWYPEVPKCEWETPEGCE**  
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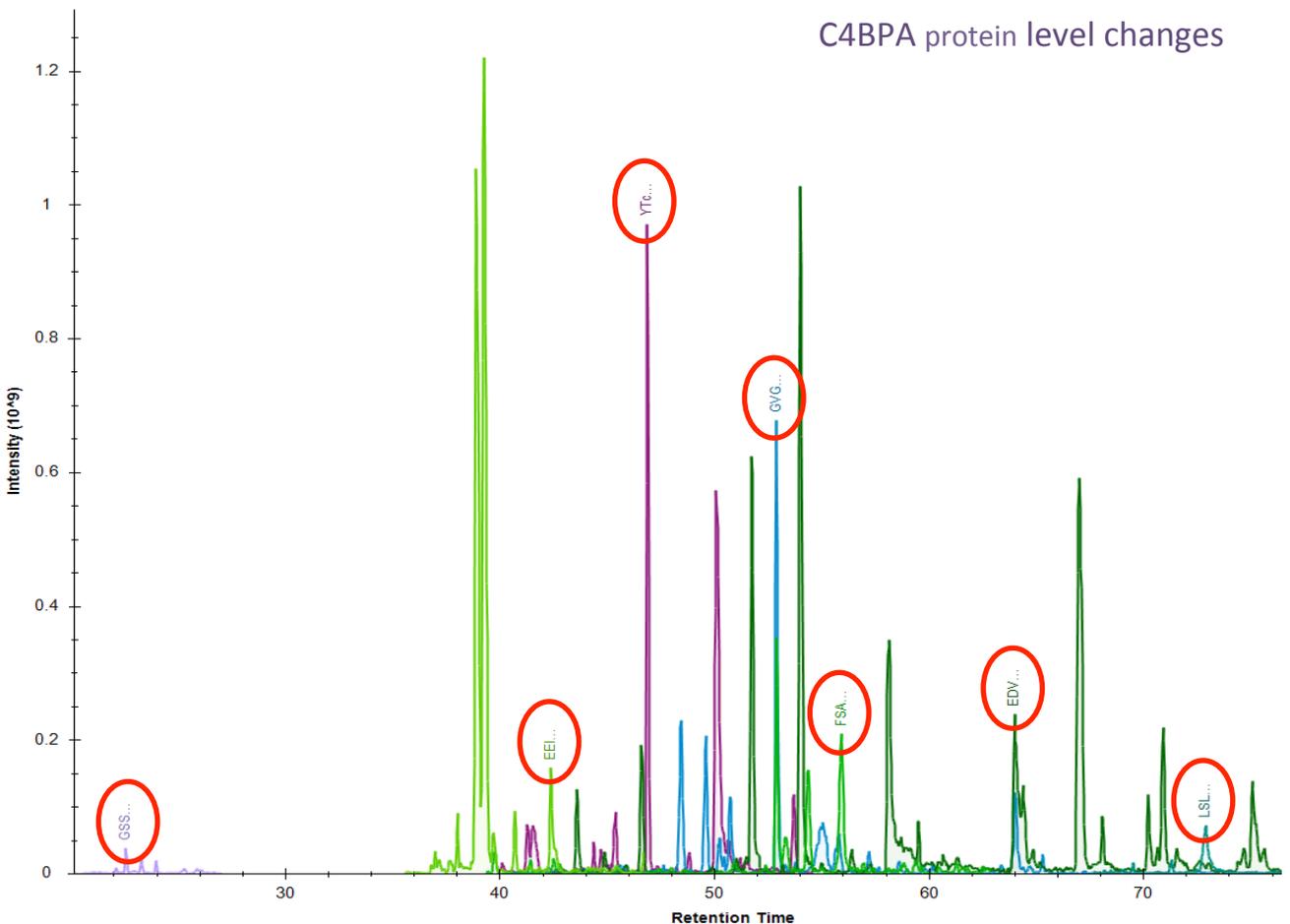
(a) Peptides quantified in control and PE subjects

- K.YT**C**LPGYVR.S [78, 86]
- R.GVGWSHPLPQ**C**EIVK.C [159, 173]
- R.GSSVIH**C**DADSK.W [274, 285]
- K.EDVYVVGTVLR.Y [318, 328]
- R.FSAI**C**QGDGTWSPR.T [404, 417]
- K.EEIIY**C**DK.G [447, 455]
- K.LSLEIEQLELQR.D [573, 584]

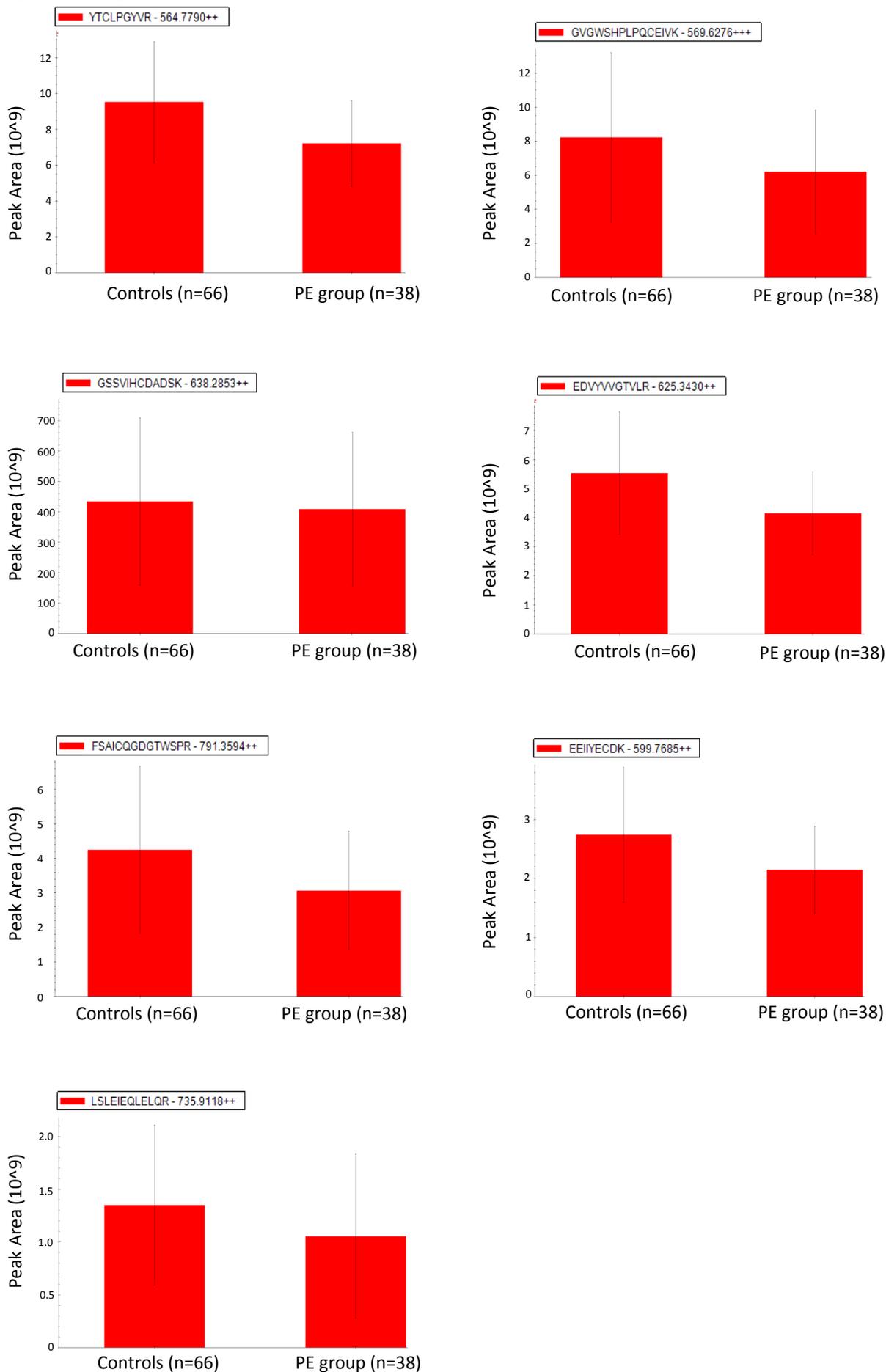


C4BPA protein level changes

(b)



**Figure 3. (a)** Total peak Area of the seven C4BPA peptides quantified in the control (n=66) and PE (n=38) groups using DIA



**Figure 3. (b)** Corresponding Peak Area - Coefficient of Variation (CV) for the seven C4BPA peptides quantified in the control (n=66) and PE (n=38) groups using DIA

