

## Supplementary Information

| Sample name       | Time post gluten intake (min) |
|-------------------|-------------------------------|
| Sample A          | 50                            |
| Sample B          | 370                           |
| Sample C          | 240                           |
| Sample D          | 90                            |
| Sample E          | 130                           |
| Pool Y (positive) | 90-230                        |
| Pool Z (negative) | -40                           |

**Supplementary Table SI1. List of duodenal fluid samples used in this study.** Samples A-E were obtained from different CeD patient samples. Pools of samples were made as described in the **Experimental** section; pool Y contained samples from six CeD patient samples; pool Z contained samples from two NCWS participants.

GDA9\_WHEAT (100 %), 35'394.6 Da

Alpha/beta-gliadin MM1 OS=Triticum aestivum OX=4565 PE=1 SV=1

7 exclusive unique peptides, 14 exclusive unique spectra, 21 total spectra, 93/307 amino acids (30 % coverage)

|                     |                      |                      |                      |                      |
|---------------------|----------------------|----------------------|----------------------|----------------------|
| M K T F L I L A L L | A I V A T T A R I A  | V R V P V P Q L Q P  | Q N P S Q Q Q P Q E  | Q V P L V Q Q Q Q F  |
| P G Q Q Q P F P P Q | Q P Y: P Q P Q P F P | S Q Q P Y L: Q L Q P | F P Q P Q L P Y: P Q | P Q L: P Y P Q P Q L |
| P Y P Q P Q P F R P | Q Q P Y P Q S Q P Q  | Y S Q P Q Q P I S Q  | Q Q Q Q Q Q Q Q Q Q  | Q K Q Q Q Q Q Q Q Q  |
| I L Q Q I L Q Q Q L | I P C R D V V L Q Q  | H S I A Y G S S Q V  | L Q Q S T Y Q L V Q  | Q L C C Q Q L W Q I  |
| P E Q S R C Q A I H | N V V H A I I L H Q  | Q Q Q Q Q Q Q Q Q Q  | Q P L S Q V S F Q Q  | P Q Q Q Y P S G Q G  |
| S F Q P S Q Q N P Q | A Q G S V Q P Q Q L  | P Q F E E I R N L A  | L E T L P A M C N V  | Y I P P Y C T I A P  |
| V G I F G T N       |                      |                      |                      |                      |

## 12&13&19&33-mers

GDBX\_WHEAT (100 %), 34'298.2 Da

Gamma-gliadin OS=Triticum aestivum OX=4565 PE=3 SV=1

2 exclusive unique peptides, 3 exclusive unique spectra, 5 total spectra, 53/302 amino acids (18 % coverage)

|                     |                     |                     |                     |                     |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| M K T L L I L T I L | A M A T T I A T A N | M Q V D P S G Q V Q | W P Q Q Q P F P Q P | Q Q P F C Q Q P Q R |
| T I P Q P H Q T F H | H Q P Q Q T F P Q P | Q Q T Y P H Q P Q Q | Q F P Q T Q Q P Q Q | P F P Q P Q Q T F P |
| Q Q P Q L P F P Q Q | P Q Q P F P Q P Q Q | P Q Q P F P Q S Q Q | P Q Q P F P Q P Q Q | Q F P Q P Q Q P Q Q |
| S F P Q Q Q Q P A I | Q S F L Q Q Q M N P | C K N F L L Q Q C N | H V S L V S S L V S | I I L P R S D C Q V |
| M Q Q Q C C Q Q L A | Q I P Q Q L Q C A A | I H S V A H S I I M | Q Q E Q Q Q G V P I | L R P L F Q L A Q G |
| L G I I Q P Q Q P A | Q L E G I R S L V L | K T L P T M C N V Y | V P P D C S T I N V | P Y A N I D A G I G |
| G Q                 |                     |                     |                     |                     |

## 15&26-mers

GDA4\_WHEAT (100 %), 34'236.1 Da

Alpha/beta-gliadin A-IV OS=Triticum aestivum OX=4565 PE=2 SV=1

2 exclusive unique peptides, 4 exclusive unique spectra, 10 total spectra, 79/297 amino acids (27 % coverage)

|                     |                      |                     |                      |                      |
|---------------------|----------------------|---------------------|----------------------|----------------------|
| M K T F L I L A L R | A I V A T T A T I A  | V R V P V P Q L Q P | Q N P S Q Q Q P Q K  | Q V P L V Q Q Q Q F  |
| P G Q Q Q P F P P Q | Q P Y: P Q Q Q P F P | S Q Q P Y M Q L Q P | F P Q P Q L P Y: P Q | P Q L: P Y P Q P Q P |
| F R P Q Q S Y P Q P | Q P Q Y S Q P Q Q P  | I S Q Q Q Q Q Q Q Q | Q Q Q Q Q Q Q I L Q  | Q I L Q Q Q L I P C  |
| R D V V L Q Q H S I | A H G S S Q V L Q Q  | STY Q L V Q Q F C   | C Q Q L W Q I P E Q  | S R C Q A I H N V V  |
| H A I I L H Q Q Q Q | Q Q Q Q Q Q Q Q Q Q  | Q P L S Q V C F Q Q | S Q Q Q Y P S G Q G  | S F Q P S Q Q N P Q  |
| A Q G S V Q P Q Q L | P Q F E E I R N L A  | L E T L P A M C N V | Y I P P Y C T I A P  | V G I F G T N        |

## 12&13&17-mers

GDBB\_WHEAT (100 %), 32'965.1 Da

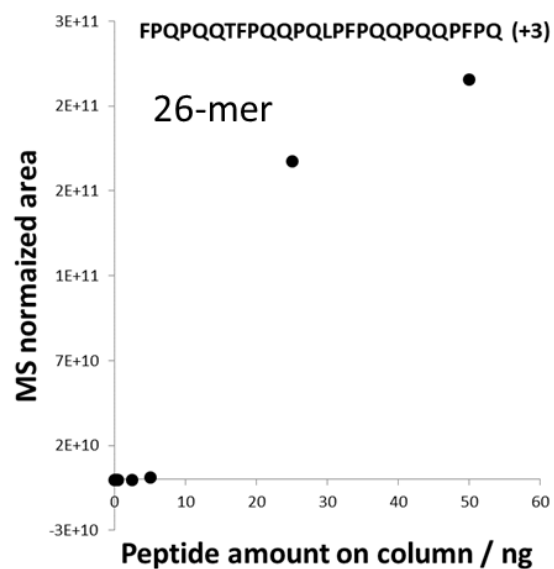
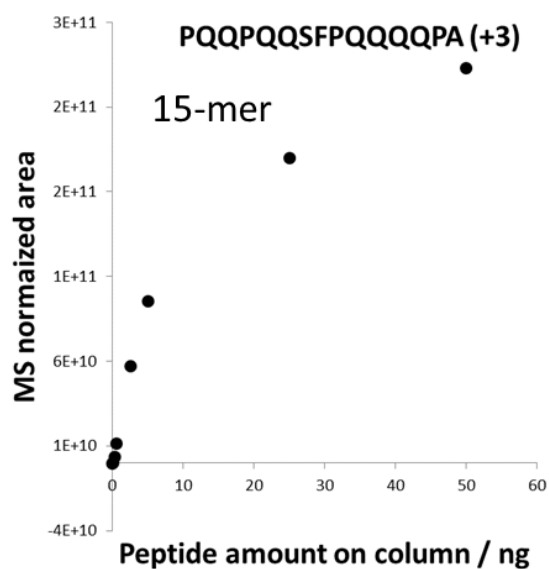
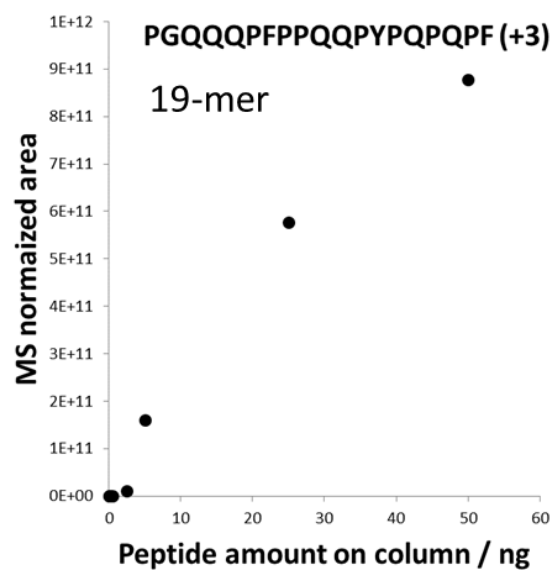
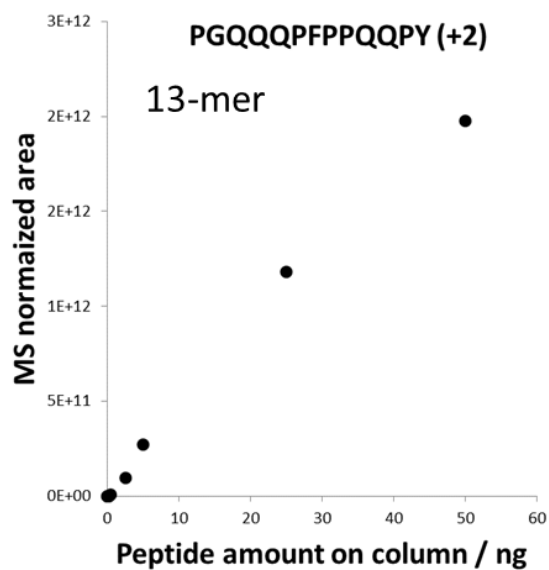
Gamma-gliadin B OS=Triticum aestivum OX=4565 PE=3 SV=1

7 exclusive unique peptides, 17 exclusive unique spectra, 33 total spectra, 135/291 amino acids (46 % coverage)

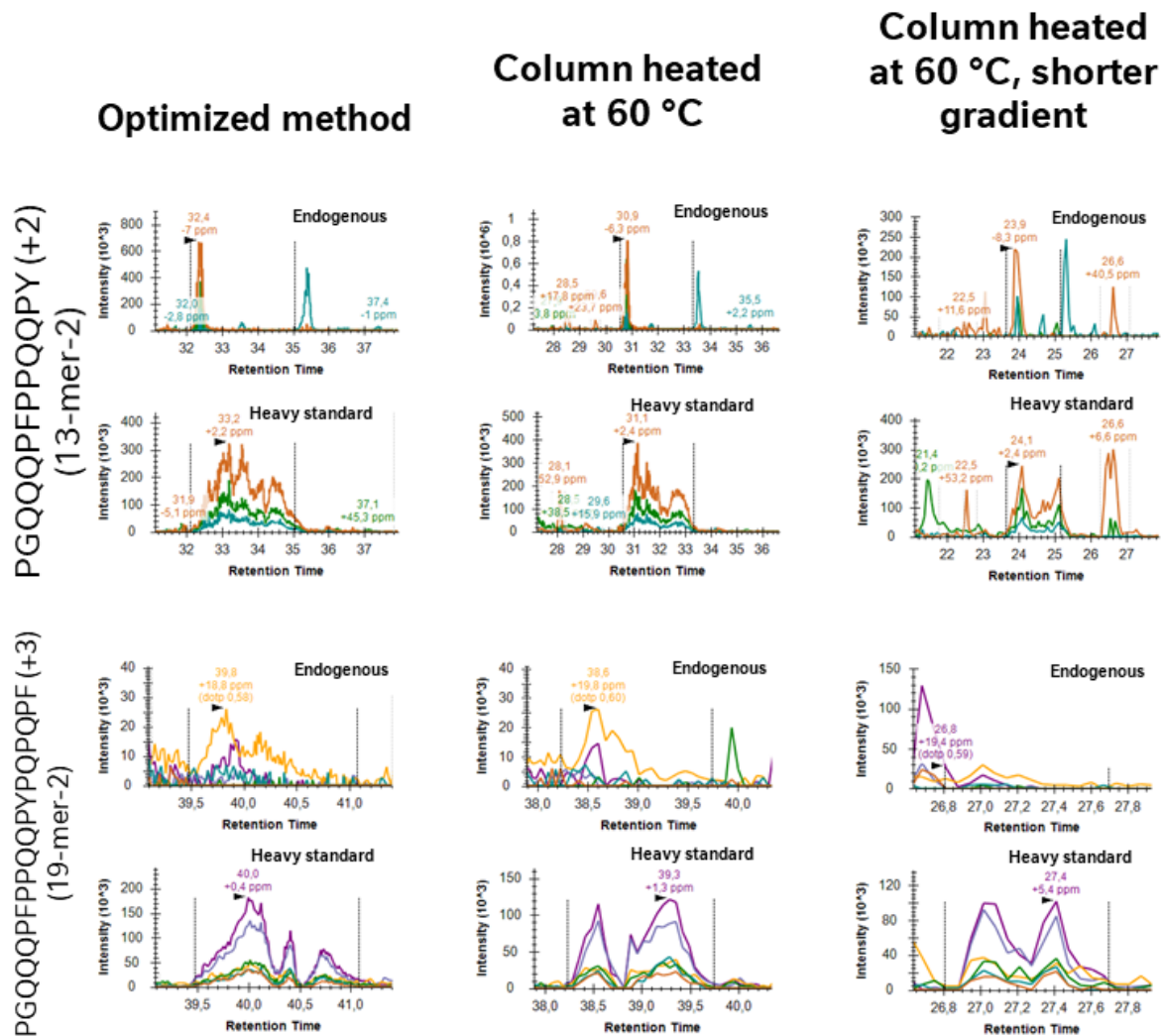
|                     |                     |                     |                     |                     |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| M K T L L I L T I L | A M A I T I A T A N | M Q A D P S G Q V Q | W P Q Q Q P F L Q P | H Q P F S Q Q P Q Q |
| I F P Q P Q Q T F P | H Q P Q Q Q F P Q P | Q Q P Q Q Q F L Q P | R Q P F P Q Q P Q Q | P Y P Q Q P Q Q P F |
| P Q T Q Q P Q Q P F | P Q S K Q P Q Q P F | P Q P Q Q P Q Q S F | P Q Q Q P S L I Q Q | S L Q Q Q L N P C K |
| N F L L Q Q C K P V | S L V S S L W S I I | L P P S D C Q V M R | Q Q C C Q Q L A Q I | P Q Q L Q C A A I H |
| S V V H S I I M Q Q | E Q Q E Q L Q G V Q | I L V P L S Q Q Q Q | V G Q G I L V Q G Q | G I I Q P Q Q P A Q |
| L E V I R S L V L Q | T L P T M C N V Y V | P P Y C S T I R A P | F A S I V A S I G G | Q                   |

## 26-mer

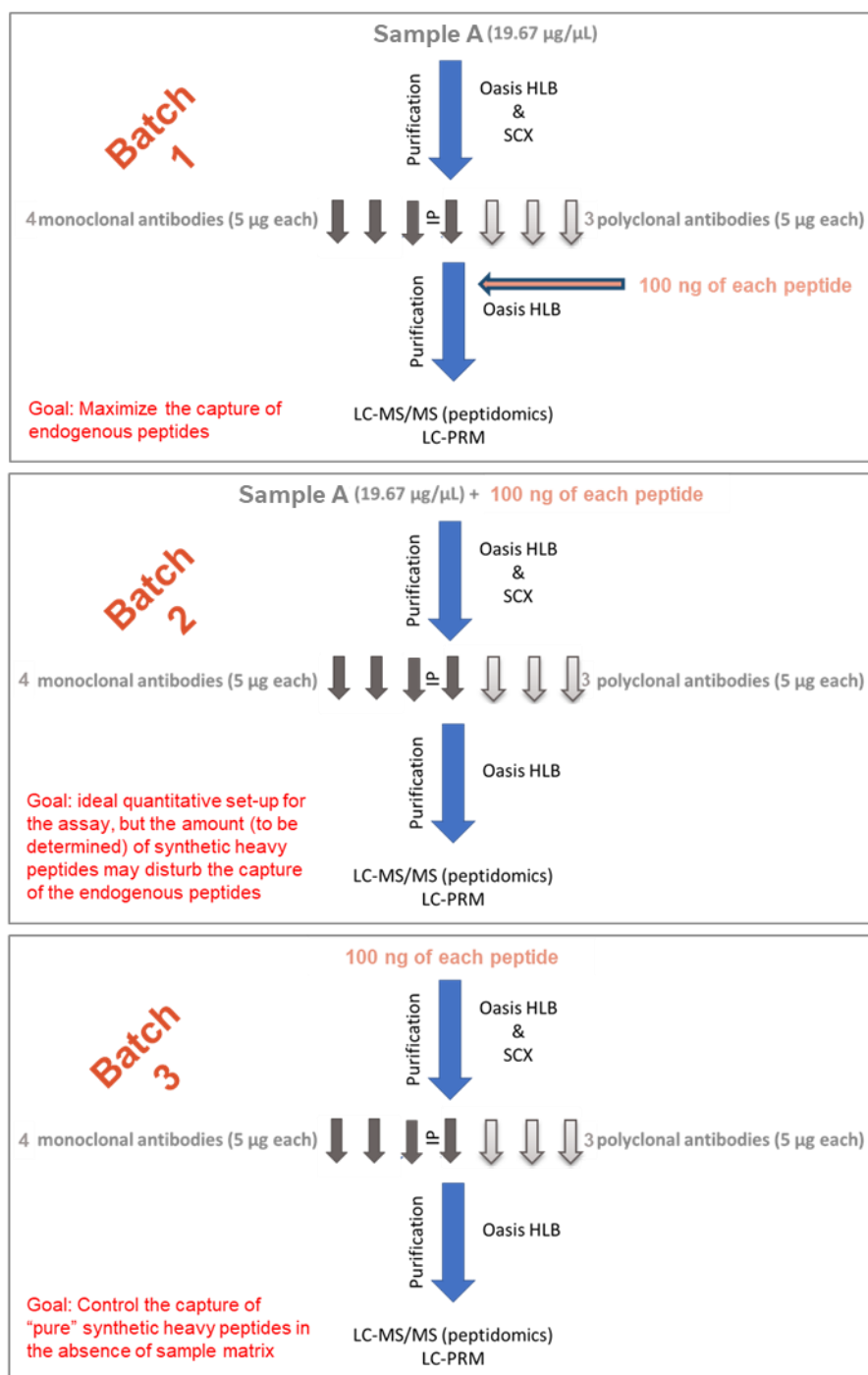
**Supplementary Figure SI1. Immunogenic amino acid sequences and identified gliadin proteins/peptides in a specific gluten product.** MS-based proteomic analysis identified nine gliadins (*i.e.*, GDB1, GDB2, GDB3, GDBB, GDBX, GDA4, GDA6, GDA7, and GDA9) in the gluten product digested with trypsin/Lys-C. Selected/targeted immunogenic amino acid sequences (highlighted and framed in blue) are known to be present in these gliadins but most of them were not overlapping with the tryptic peptide sequences (in yellow) detected. Modified amino acids are highlighted in green.



**Supplementary Figure SI2. Quantitative response of the isotopically labelled gluten peptides in a matrix-free buffer using LC-PRM-MS.** LC-PRM-MS targets the doubly charged ion of 13-mer, the triply charged ion of 19-mer, the triply charged ion of 15-mer, and the triply charged ion of 26-mer.

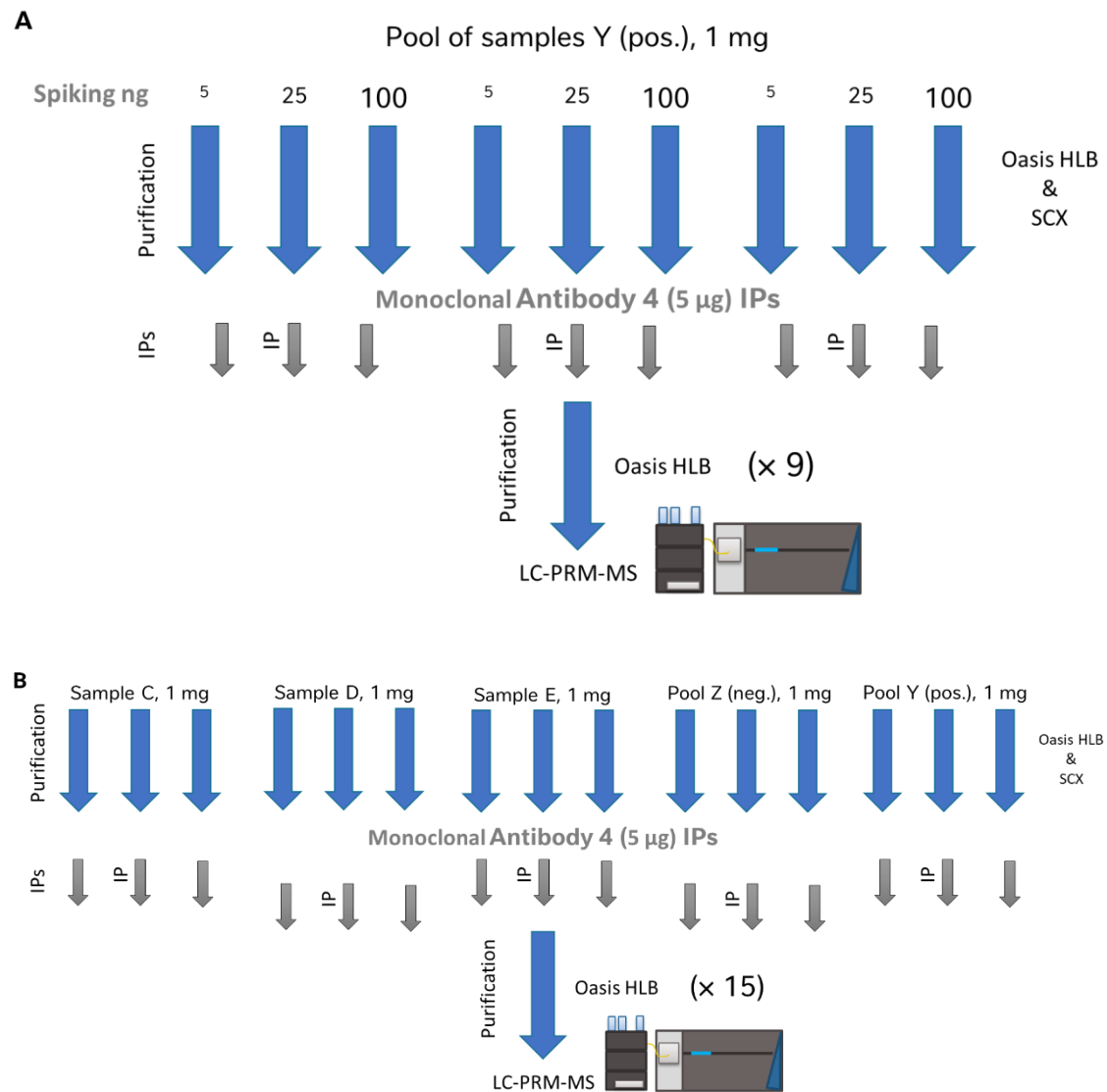


**Supplementary Figure S13. LC chromatogram obtained for 13-mer and 19-mer peptides using LC-PRM-MS.** Recorded fragment-ion signals correspond to the detection of selected fragments for both the endogenous and isotopically labeled peptides. Despite some LC condition optimization attempts, RP-LC elution peaks continued to exhibit splitting.



**Supplementary Figure S14. IP procedure evaluation before LC-MS/MS.** Different experimental schemes were followed to assess the IP of gluten peptides endogenously present in duodenal fluids and spiked as isotopically labelled standards in the samples. Immuno-enriched fractions obtained with each of the seven tested antibodies were analysed with LC-MS/MS using both DDA and PRM modes of acquisition.





**Supplementary Figure SI6. Scheme of the experimental design followed to optimize and qualify the measurement of gluten peptides in duodenal fluid samples.** A first trial assessed the optimal amount of isotopically labeled standards to be spiked (**A**). The second trial aimed at assessing the reproducibility of the measurements in different samples (using the determined optimal amount of 5 ng of isotopically labeled standards). Measurements were performed with two identical but independent LC-MS/MS systems, at two different days (see also **Figure 4**) (**B**).