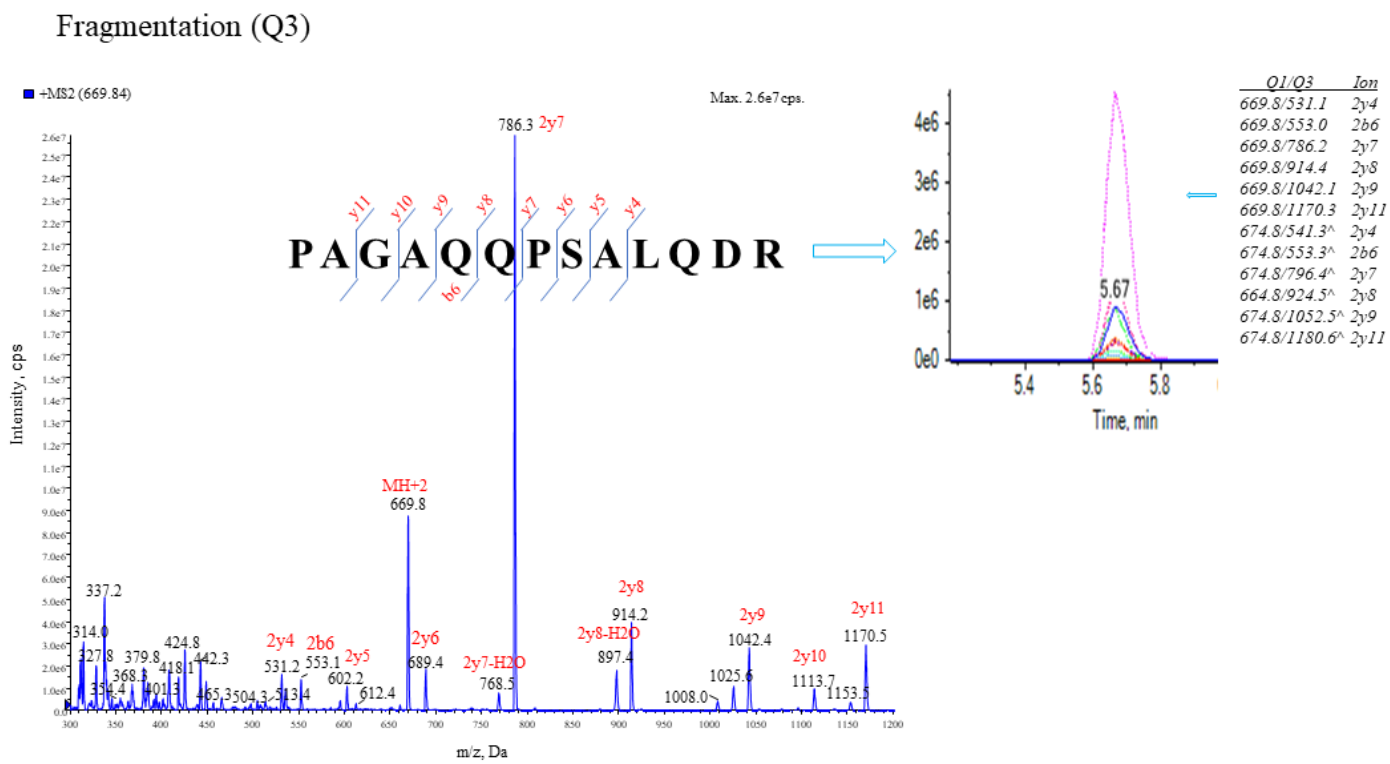
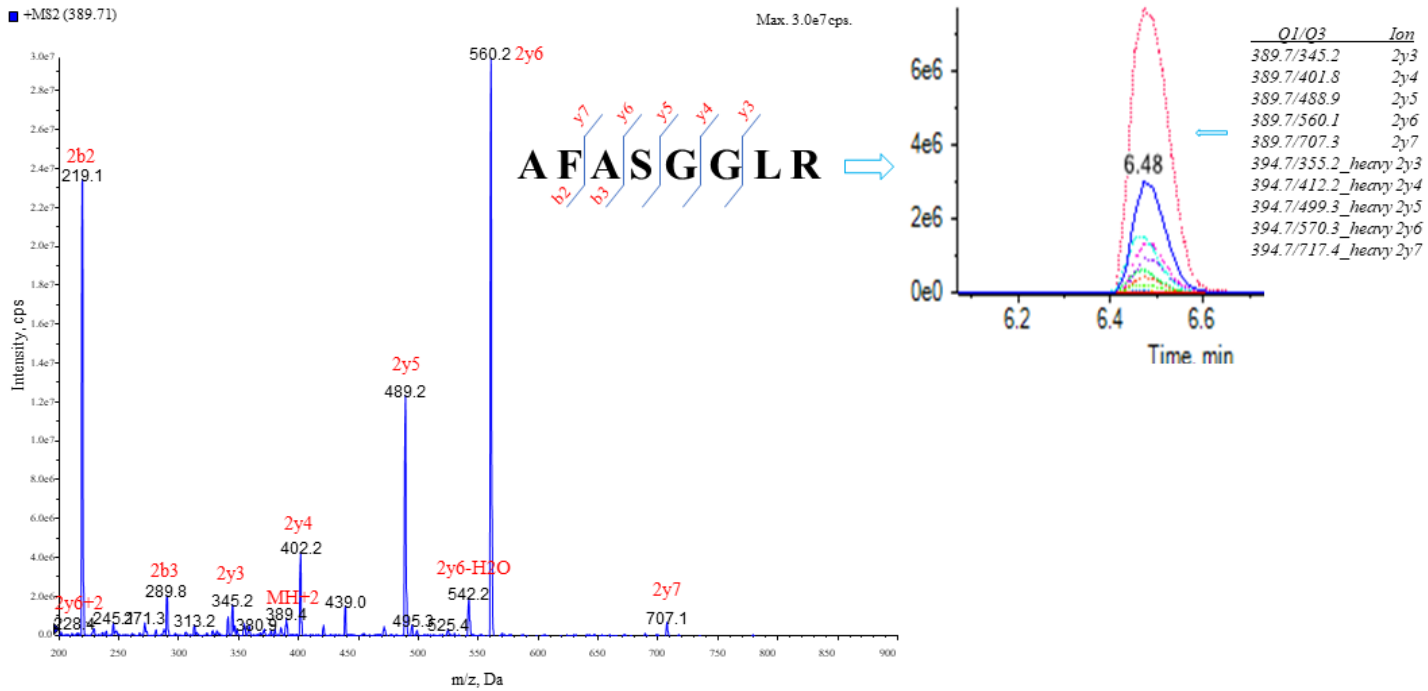
Supplemental Fig 1A




Supplemental Fig 1A: The MS/MS spectra of the peptide pep-U1. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1B

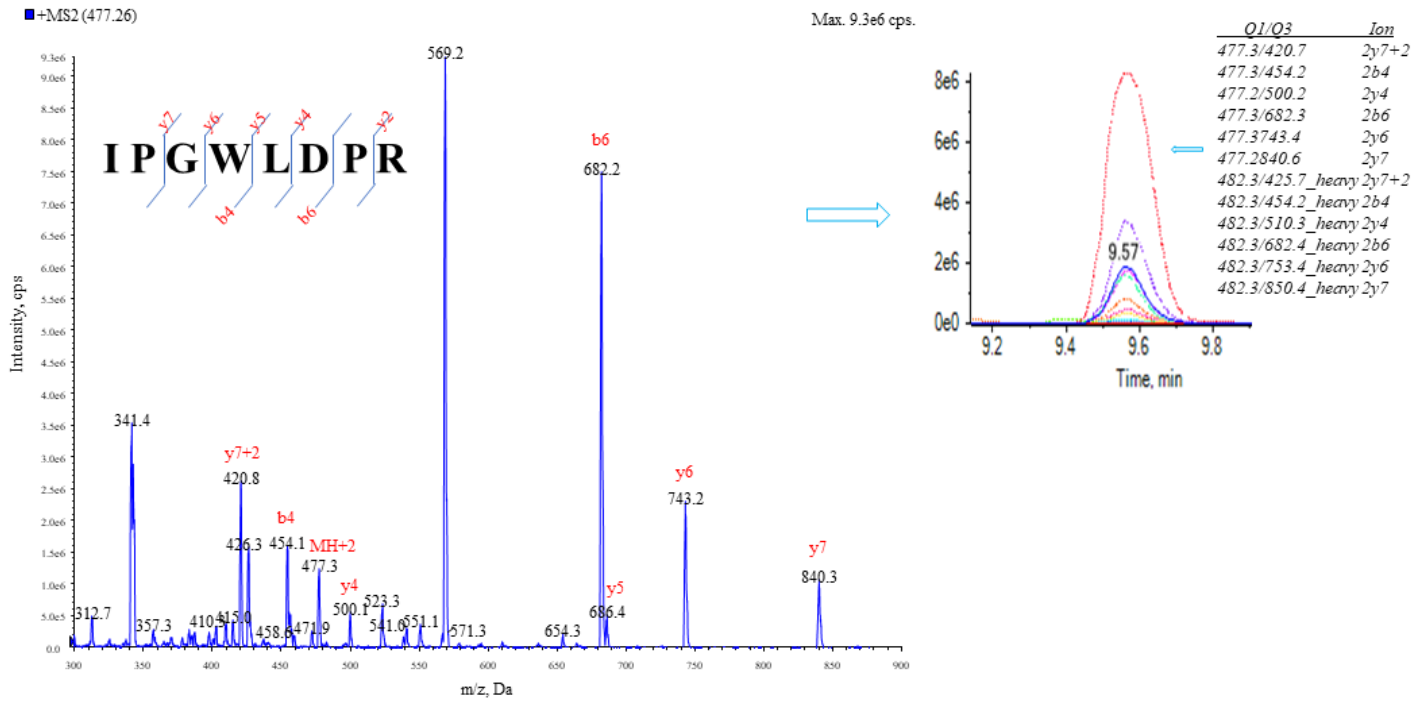
Fragmentation (Q3)




Supplemental Fig 1B: The MS/MS spectra profile of the peptide pep-U2. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1C

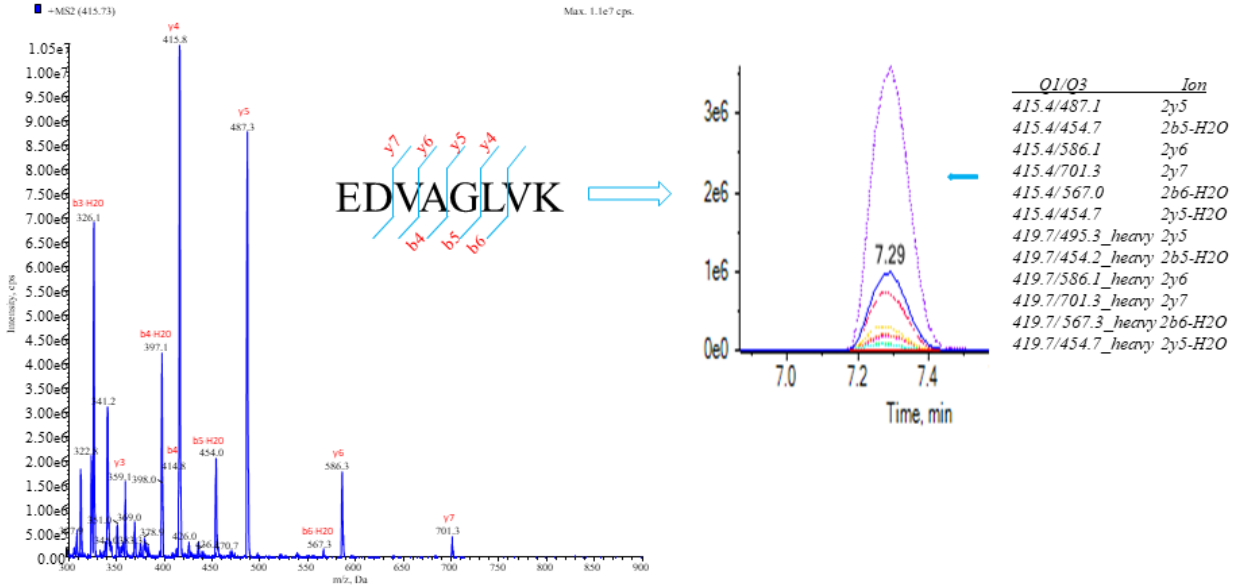
Fragmentation (Q3)




Supplemental Fig 1C: The MS/MS spectra of the peptide pdp-U3. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1D

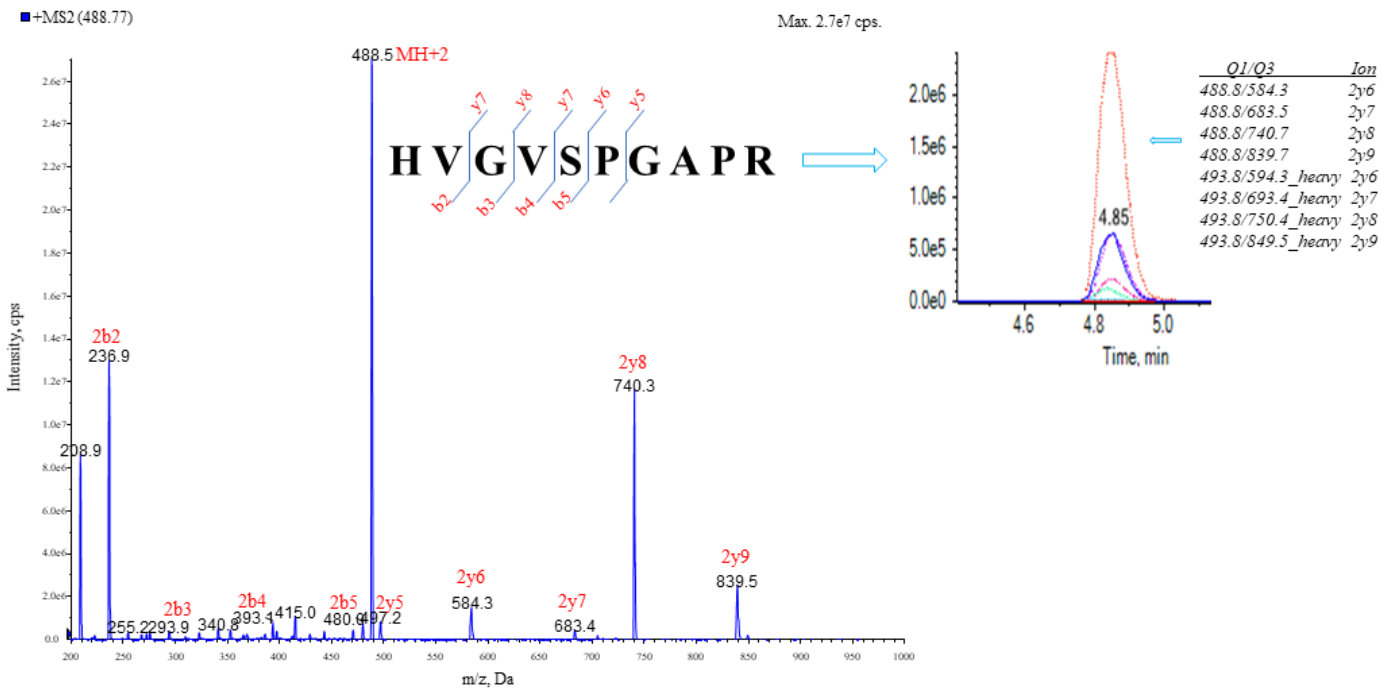
Fragmentation (Q3)




Supplemental Fig 1D: The MS/MS spectra of the peptide pep-U4. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

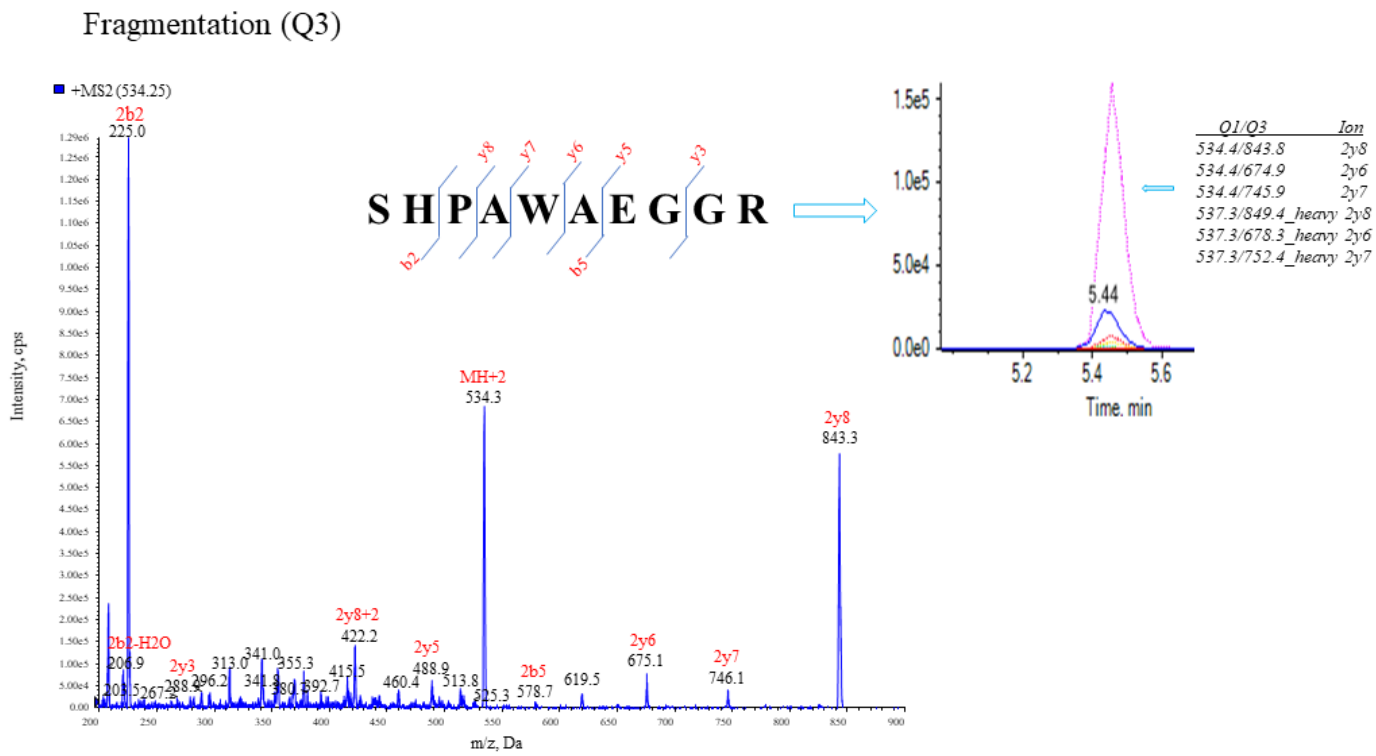
Supplemental Fig 1E

Fragmentation (Q3)



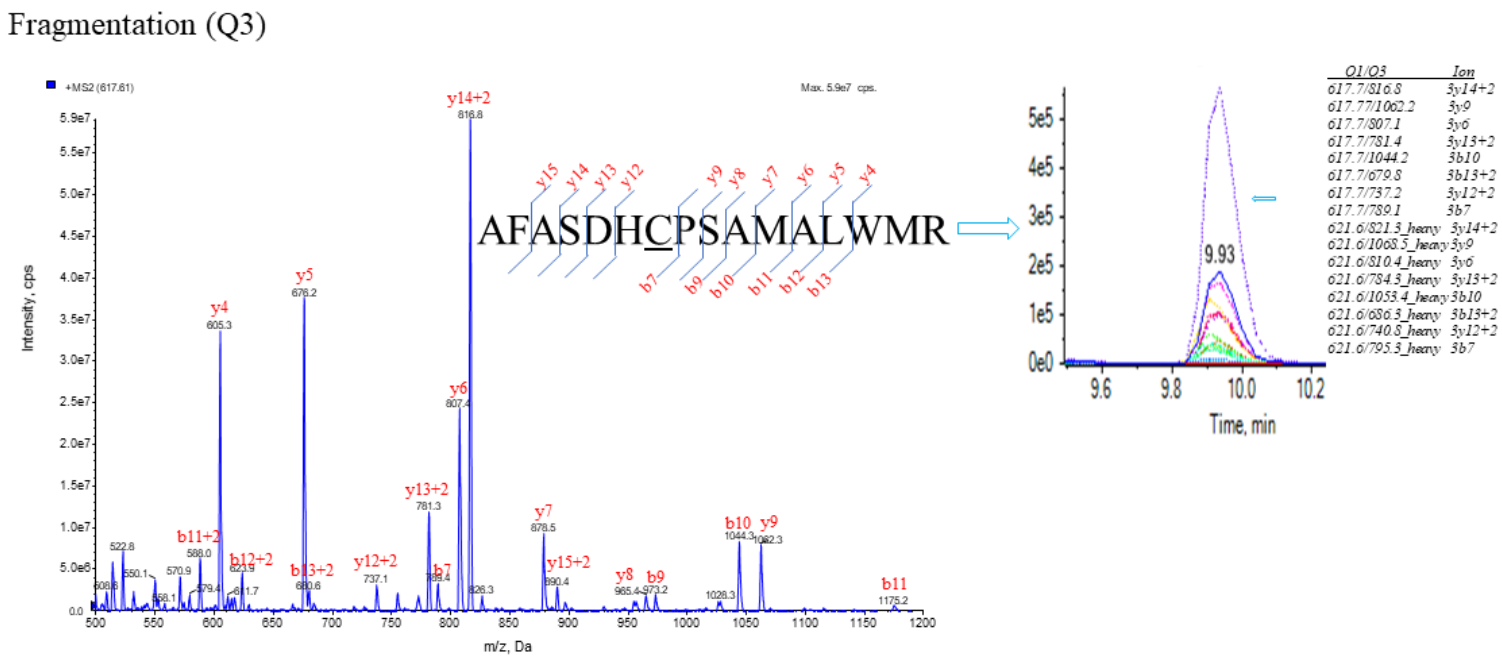

Supplemental Fig 1E: The MS/MS spectra of the peptide pep-U5. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1F



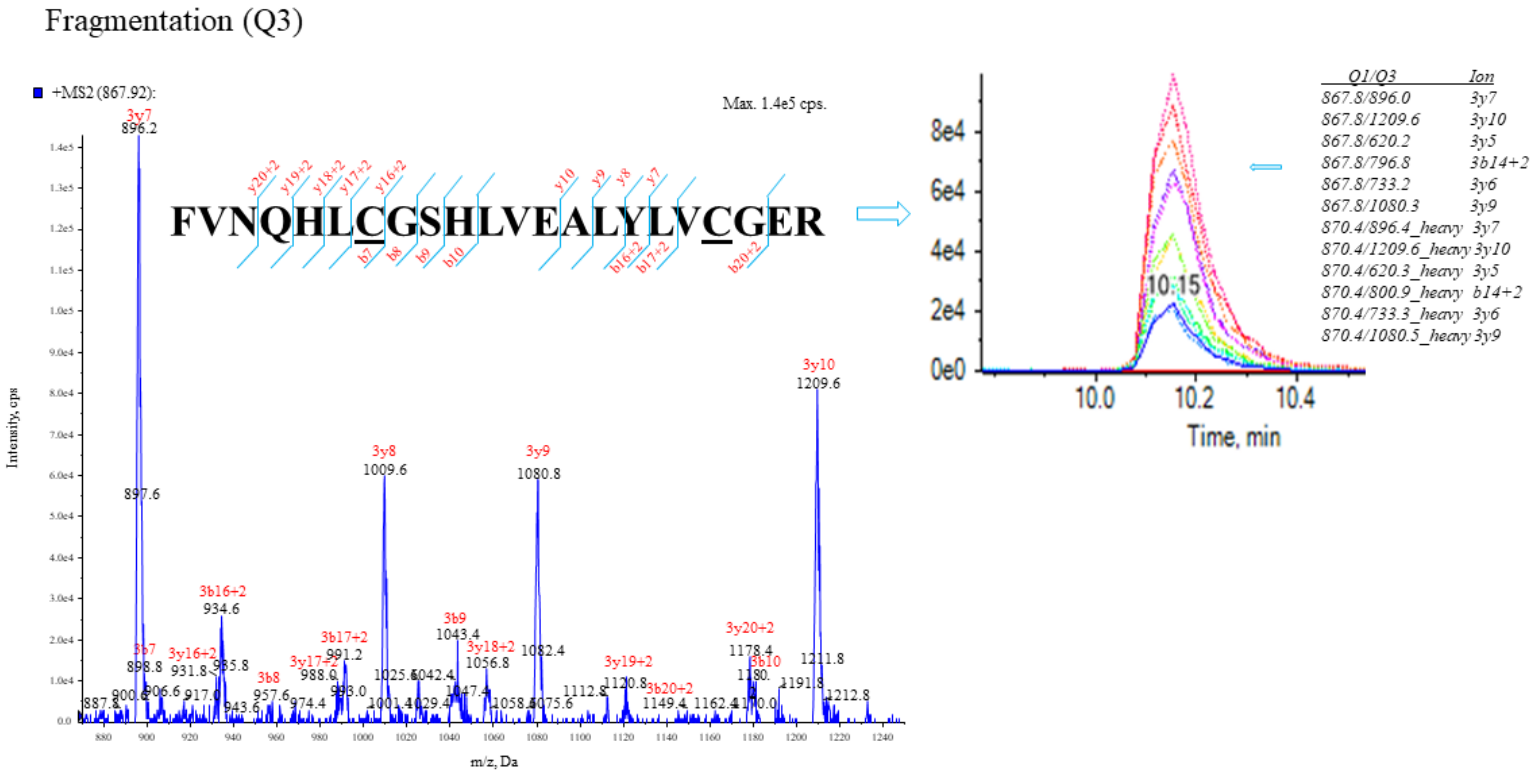

Supplemental Fig 1F: The MS/MS spectra of the peptide pep-UF. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1G




Supplemental Fig 1G: The MS/MS spectra of the peptide pep-US. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present. Underlined cysteine residues denote carbamidomethylation (Cys-CAM).

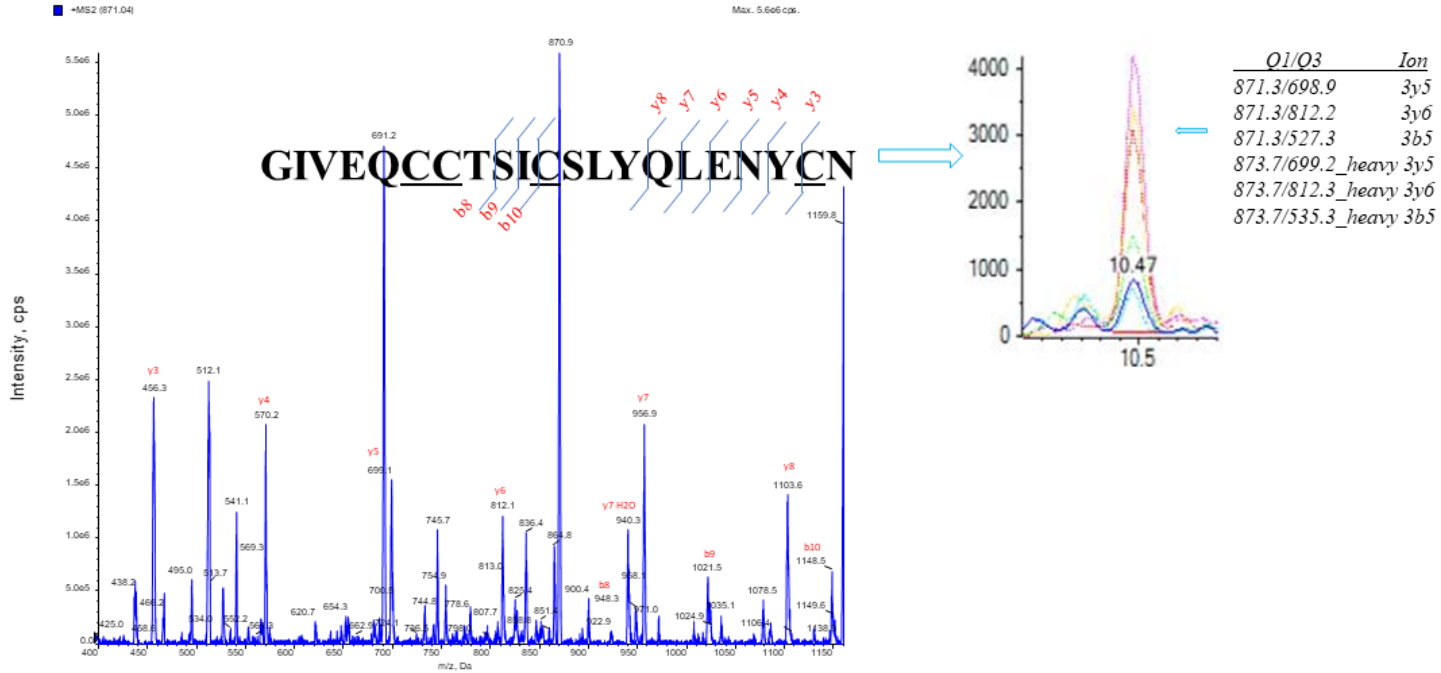
Supplemental Fig 1H




Supplemental Fig 1H: The MS/MS spectra of the target peptide pep-B. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present. Underlined cysteine residues denote carbamidomethylation (Cys-CAM).

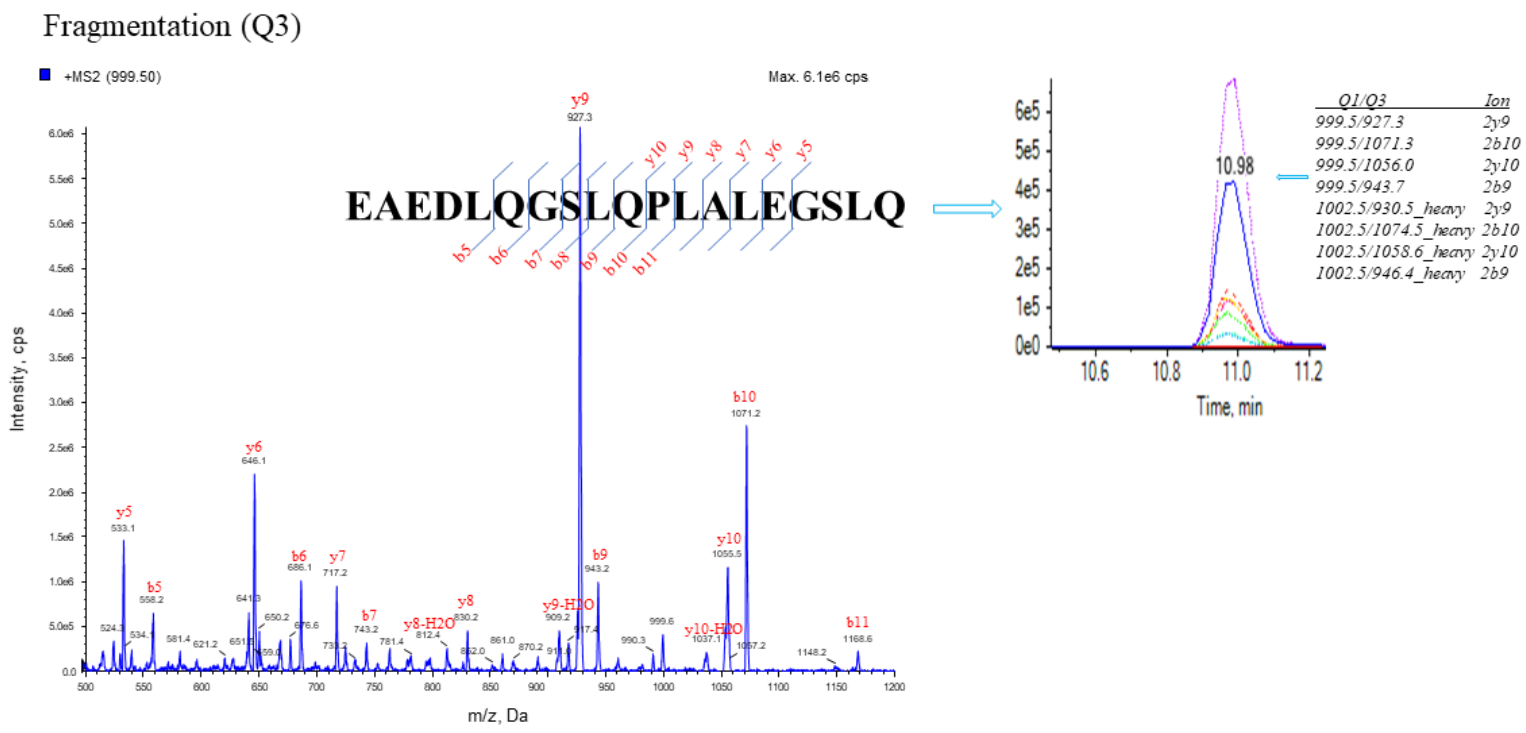
Supplemental Fig 1I

Fragmentation (Q3)



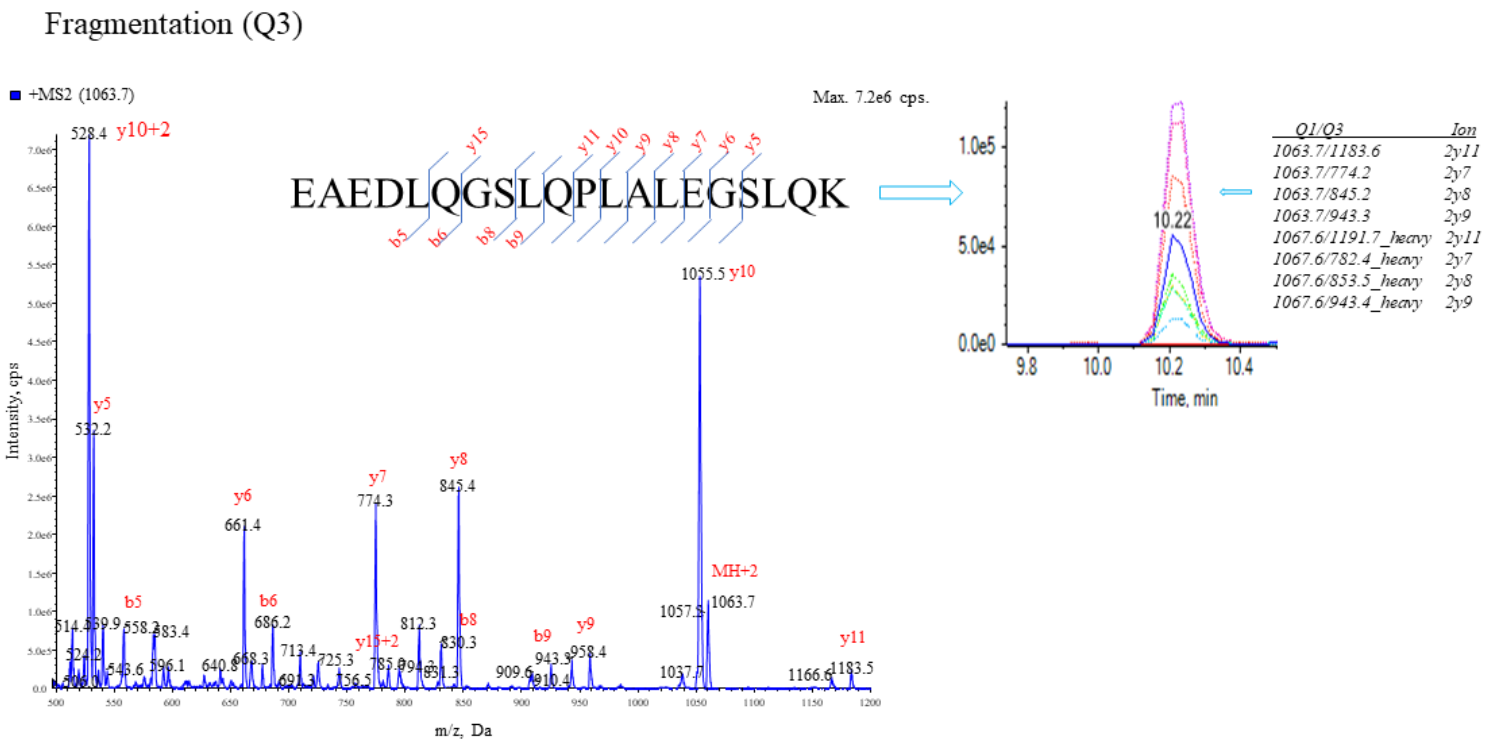

Supplemental Fig 1I: The MS/MS spectra of the peptide pep-A. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present. Underlined cysteine residues denote carbamidomethylation (Cys-CAM).

Supplemental Fig 1J




Supplemental Fig 1J: The MS/MS spectra of the peptide PEP-Cα. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 1K




Supplemental Fig 1K: The MS/MS spectra profile of the peptide Pep-CαK. Insert: a set of coeluting transition peaks in plasma matrix confirm that the detected SRM signals do derive from the fragment peptide b and y ions and no interfering of co-eluting peaks were present.

Supplemental Fig 2A

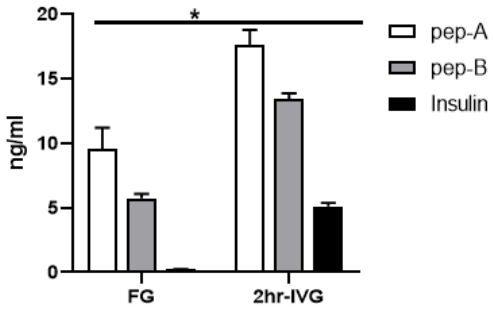
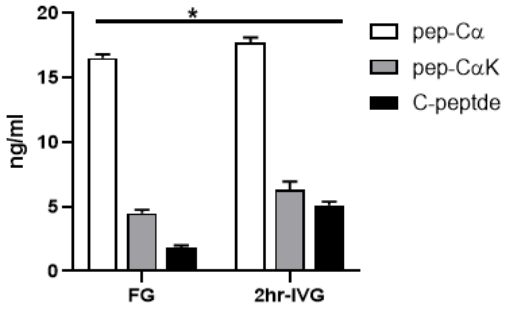



Fig 2B




Supplemental Fig 2: (A) Quantification by SRM-MS of pep-A (A-chain), pep-B (B-chain) and (total) insulin by ELISA; (B) quantification by SRM-MS of processed pep-C (alpha) and non-processed pep-CaK, and C-peptide by ELISA; Plasma was obtained after an overnight fast (FG) and after 2hr-IVG 2-hour continuous intravenous glucose infusion (2 hours: fasting glucose + 98 mg glucose). Asterisk represents significant differences by 2-way ANOVA.

Supplemental Fig 3A

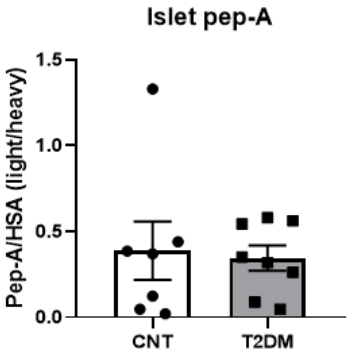



Fig 3B

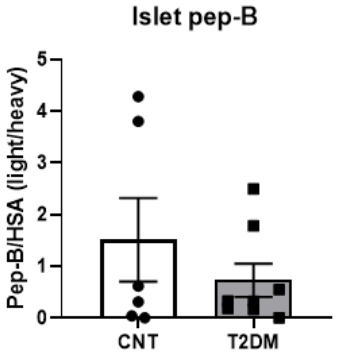
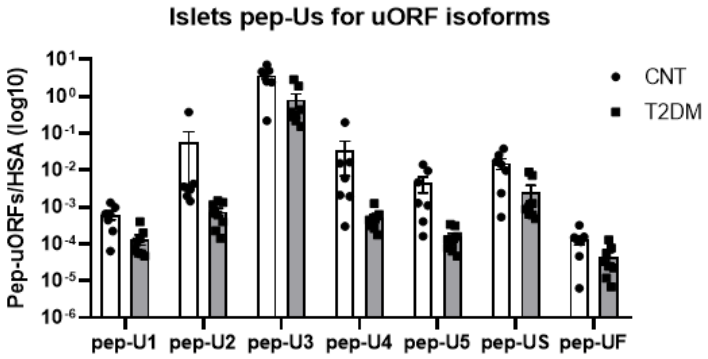



Fig 3C




Supplemental Fig 3: SRM –MS quantification of A-chain (A) and B-chain (B) in human islets of control (CNT) and T2DM samples. Y axis represents endogenous pep-A and pep-B transition peak areas normalized with those of respective isotope-labeled tryptic peptides.

Supplemental Fig 4A

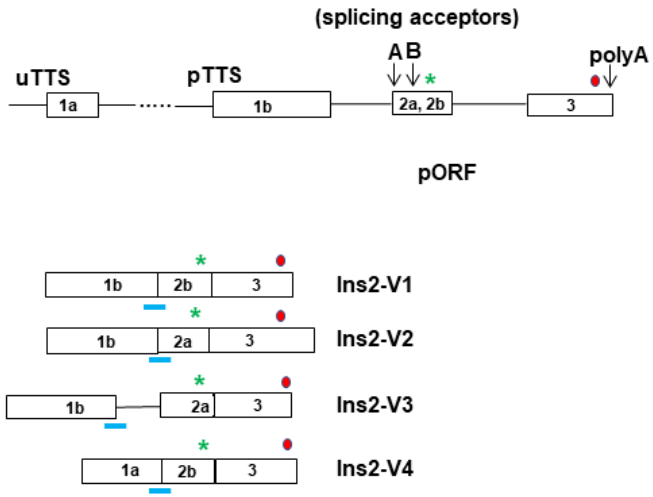
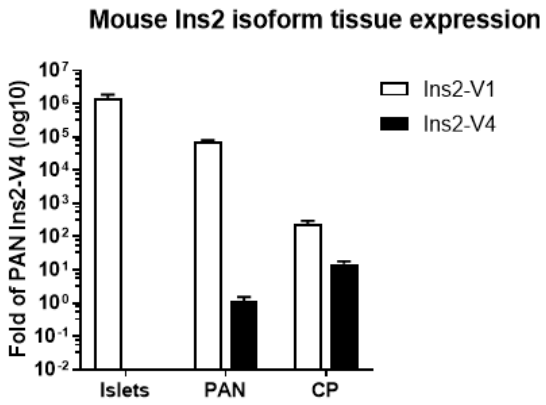



Fig 4B




Supplemental Figure 4: (A): mouse *Ins2* gene structures and their alternatively spliced isoforms. uTSS, upstream transcription start site; pTSS, primary transcription start site. Open boxes represent exons and solid lines represent introns. Downward arrows and capital letters are at exon-2 and polyadenylation sites. Green asterisk represents translation initiation codon and red dot stop codon. (B): TaqMan RT-qPCR of mouse Ins2 uTSS and pTSS isoforms, Ins2-V1 and Ins2-V4, in islets, PAN (pancreas), and CP (choroid plexus). Ins2-V1 isoform mRNA was found in mouse islets to be more than $10^5$-fold higher than in CP, and $10^8$-fold higher than Ins2-V4.

**Supplemental Table 1: Checklist for Reporting Human Islet Preparations Used in Research**

| | |
|---|---|
| **Manuscript DOI:** https://doi.org/10.2337/ **[insert manuscript submission number]** (Example, https://doi.org/10.2337/db18-1234) | |
| **Title: Novel human insulin isoforms and a short Cα-peptide product in islets of Langerhans and choroid plexus** | |
| **Author list:** Qing-Rong Liu, Min Zhu, Pingbo Zhang, Caio H. Mazucanti, Nicholas S. Huang, Doyle L. Lang, Qinghua Chen, Pavan Auluck, Stefano Marenco, Jennifer F. O'Connell, Luigi Ferrucci, Chee W. Chia, and Josephine M. Egan | |
| **Corresponding author:** Josephine M. Egan | **Email address: eganj@grc.nia.nih.gov** |

| **Islet preparation** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8[a]** |
|---|---|---|---|---|---|---|---|---|
| **MANDATORY INFORMATION** | | | | | | | | |
| Unique identifier | SAMN08768754 | SAMN08768758 | SAMN08768773 | SAMN08768767 | SAMN08768798 | SAMN08768795 | SAMN08768794 | SAMN08768792 |
| Donor age (years) | 41 | 37 | 51 | 35 | 46 | 37 | 39 | 41 |
| Donor sex (M/F) | M | F | M | M | F | M | M | F |
| Donor BMI ($kg/m^2$) | 28.5 | 19.7 | 35.6 | 31.5 | 21.5 | 22.9 | 31.9 | 19.1 |
| Donor $HbA_{1c}$ or other measure of blood glucose control | 165.00 mg/dl | 122.40 mg/dl | 187.80 mg/dl A1c=7.1 | 100.60 mg/dl | 136.20 mg/dl | 96.80 mg/dl | 125.00 mg/dl | 230.00 mg/dl |
| Origin/source of islets[b] | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Islet isolation centre | The Scharp-Lacy Research Institute | The Scharp-Lacy Research Institute | The Scharp-Lacy Research Institute | The Scharp-Lacy Research Institute | University of Wisconsin | University of Pennsylvania | University of Miami | University of Pennsylvania |
| Donor history of diabetes? Yes/No | No | No | Yes | No | No | No | No | No |
| **If Yes, complete the next two lines if this information is available** | | | | | | | | |
| Diabetes duration (years) | | | 0-5 years | | | | | |
| Glucose-lowering therapy at time of death[c] | | | Oral medication | | | | | |

| **RECOMMENDED INFORMATION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Donor cause of death | Head trauma | Anoxia | Stroke | Head trauma | Stroke | Anoxia | Stroke | Head trauma |
| Warm ischaemia time (h) | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cold ischaemia time (h) | 12.8 | 6.2 | 9.9 | 14.5 | 4.2 | 7.4 | 12.0 | 6.9 |
| Estimated purity (%) | 95% | 95% | 90% | 90% | 95% | 95% | 80% | 94% |
| Estimated viability (%) | 95% | 95% | 95% | 95% | 95% | 90% | 93% | 94% |
| Total culture time (h)[d] | 38 | 82 | 95 | 96 | 48 | 22 | 96 | 42 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Glucose-stimulated insulin secretion or other functional measurement[e] | 3.9 SI | NA | NA | NA | NA | NA | NA | NA |
| Handpicked to purity? Yes/No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Additional notes | | | | | | | | |

[a]If you have used more than eight islet preparations, please complete additional forms as necessary

[b]For example, IIDP, ECIT, Alberta IsletCore

[c]Please specify the therapy/therapies

[d]Time of islet culture at the isolation centre, during shipment and at the receiving laboratory

[e]Please specify the test and the results

| **Islet preparation** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|
| **MANDATORY INFORMATION** | | | | | | | | |
| Unique identifier | SAMN08768790 | SAMN08768788 | SAMN08768784 | SAMN08768979 | SAMN08768974 | SAMN08768973 | SAMN08768971 | SAMN08768972 |
| Donor age (years) | 51 | 46 | 52 | 59 | 28 | 45 | 61 | 27 |
| Donor sex (M/F) | F | F | F | M | M | M | M | M |
| Donor BMI (kg/m$^2$) | 22.5 | 35.9 | 42.8 | 27.7 | 29.2 | 36.4 | 27.0 | 30 |
| Donor $HbA_{1c}$ or other measure of blood glucose control | 179.60 mg/dl | 262.40 mg/dl A1c=6.8 | 237.40 mg/dl A1c=6.6 | 199.80 mg/dl A1c=6.5 | 232.00 mg/dl | 174.00 mg/dl | 146.40 mg/dl | 108.00 mg/dl |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Origin/source of islets[b] | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP |
| Islet isolation centre | The Scharp-Lacy Research Institute | University of Pennsylvania | The Scharp-Lacy Research Institute | The Scharp-Lacy Research Institute | University of Wisconsin | University of Wisconsin | Southern California Islet Cell Resource Center | University of Wisconsin |
| Donor history of diabetes? Yes/No | No | Yes | Yes | Yes | No | No | No | No |
| **If Yes, complete the next two lines if this information is available** | | | | | | | | |
| Diabetes duration (years) | | 0-5 years | 0-5 yeas | 6-10 years | | | | |
| Glucose-lowering therapy at time of death[c] | | Oral medication | Oral medication | Oral medication | | | | |

| **RECOMMENDED INFORMATION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Donor cause of death | Stroke | Stroke | Stroke | Stroke | Head trauma | Stroke | Head trauma | Head trauma |
| Warm ischaemia time (h) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.53 |
| Cold ischaemia time (h) | 11.3 | 6.9 | 6.9 | 6.1 | 3.5 | 9 | Not documented | 4.3 |
| Estimated purity (%) | 95% | 50% | 90% | 85% | 85% | 90% | 80% | 95% |
| Estimated viability (%) | 95% | 92% | 95% | 95% | 98% | 93% | 98% | 99% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Total culture time (h)[d] | 111 | 18 | 68 | 59 | 66 | 24 | 70 | 43 |
| Glucose-stimulated insulin secretion or other functional measurement[e] | NA | NA | NA | NA | NA | NA | NA | NA |
| Handpicked to purity? Yes/No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Additional notes | | | | | | | | |

| **Islet preparation** | **17** | **18** | **19** | **20** | **21** | **22** | | |
|---|---|---|---|---|---|---|---|---|
| **MANDATORY INFORMATION** | | | | | | | | |
| Unique identifier | SAMN08768992 | SAMN08769032 | SAMN08769026 | SAMN08616281 | SAMN08617637 | SAMN10229738 | | |
| Donor age (years) | 16 | 26 | 37 | 45 | 54 | 46 | | |
| Donor sex (M/F) | M | M | F | M | M | F | | |
| Donor BMI (kg/m$^2$) | 31.5 | 28.7 | 38.1 | 27.2 | 24.5 | 33.2 | | |
| Donor $HbA_{1c}$ or other measure of blood glucose control | 209.20 mg/dl | 142.00 mg/dl A1c=6.5 | 253.80 mg/dl A1c=8.2 | 246.00 mg/dl A1c=6.5 | 122.40 mg/dl A1c=4.8 | 164.20 mg/dl A1C=10.7 | | |
| Origin/source of islets[b] | IIDP | IIDP | IIDP | IIDP | IIDP | IIDP | | |
| Islet isolation centre | University of Pennsylvania | The Scharp-Lacy Research Institute | University of Pennsylvania | University of Wisconsin | University of Wisconsin | Southern California Islet Cell Resources Center | | |
| Donor history of diabetes? Yes/No | No | Yes | Yes | Yes | Yes | Yes | | |
| **If Yes, complete the next two lines if this information is available** | | | | | | | | |
| Diabetes duration (years) | | 0-5 years | 0-5 yeas | 0-5 years | 6 years | >10 years | | |
| Glucose-lowering therapy at time of death[c] | | Oral medication | Oral medication | Oral medication | Oral medication | Insulin 20 units | | |

| RECOMMENDED INFORMATION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Donor cause of death | Anoxia | Head trauma | Stroke | Stroke | Stroke | Stroke | | |
| Warm ischaemia time (h) | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Cold ischaemia time (h) | 9.4 | 7.3 | 4.7 | 11.4 | 10.1 | 5.8 | | |
| Estimated purity (%) | 70% | 90% | 85% | 85% | 80% | 95% | | |
| Estimated viability (%) | 95% | 95% | 94% | 95% | 92% | 95% | | |
| Total culture time (h)[d] | 21 | 55 | 22 | 101 | 18 | 30 | | |
| Glucose-stimulated insulin secretion or other functional measurement[e] | NA | NA | NA | NA | NA | NA | | |
| Handpicked to purity? Yes/No | Yes | Yes | Yes | Yes | Yes | Yes | | |
| Additional notes | | | | | | | | |

**Supplemental Table 2**: Clinical data of human postmortem choroid plexus (PMI: postmortem interval; RIN: RNA integrity number; BMI: body mass index; ASCVD: Arteriosclerotic Hypertensive Cardiovascular Disease; COPD: Chronic Obstructive Pulmonary Disease; ND: No Diagnosis).

| Age | Sex | Race | PMI | Ph | RIN | Smoker | BMI | Axis III | Cause of Death |
|---|---|---|---|---|---|---|---|---|---|
| 35.9 | Male | African American | 49.5 | 5.88 | 4.2 | No | 19.1 | Diabetes Mellitus, Type I, (IDDM), N.D. | THREE VESSEL OCCLUSIVE CORONARY ATHEROSCLEROSIS |
| 56.3 | Male | African American | 31.5 | 6.09 | NA | Unknown | 31.3 | Diabetes Mellitus, Type I, (IDDM), N.D. | PULMONARY EMBOLISM DUE TO DEEP VENOUS THROMBOSIS |
| 61.2 | Female | Caucasian | 24.5 | 5.99 | 5.9 | No | 31.4 | Diabetes Mellitus, Type I, (IDDM) N.D. | CORONARY ARTERIOSCLEROSIS |
| 48.5 | Female | African American | 23.5 | 6.25 | 5.9 | Unknown | 26.9 | Diabetes Mellitus, Type I, (IDDM), Hypertension | SEVERE GENERALIZED ATHEROSCLEROSIS DUE TO HYPERTENSION |
| 41.5 | Male | Caucasian | 34.5 | 6.47 | 8.4 | No | 27.2 | Diabetes Mellitus, Type I, (IDDM), ASHCVD | ATHEROSCLEROTIC CARDIOVASCULAR DISEASE |
| 84.2 | Male | African American | 27.5 | 6.27 | NA | No | 41.2 | Diabetes Mellitus, Type I, (IDDM), COPD | MULTIPLE BLUNT IMPACT INJURIES |
| 41.1 | Male | African American | 55.5 | 6.73 | NA | No | 24.1 | Diabetes Mellitus, Type II (Non-IDDM), Hypertension | HYPERTENSIVE CARDIOVASCULAR DISEASE WITH BIVENTRICULAR DIALA |
| 41.2 | Female | African American | 32 | 6.62 | 8.9 | Yes | 27.4 | Diabetes Mellitus, Type II (Non-IDDM), Hypertension | ASHCVD WITH CORONARY ARTERY STENOSIS |
| 54.9 | Male | African American | 27 | 6.11 | 8.4 | No | 42.1 | Diabetes Mellitus, Type II (Non-IDDM), Hypertension | PULMONARY EBLOLUS DUE TO DEEP VENOUS THROMBOSIS |
| 56.5 | Male | African American | 24 | 6.51 | 8.9 | No | 40.3 | Diabetes Mellitus, Type II (Non-IDDM), Hypertension | PULMONARY EBLOLUS DUE TO DEEP VENOUS THROMBOSIS |
| 27.6 | Female | African American | 21 | 6.41 | 8.3 | No | 52 | Non-Diabetes, Obesity | ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA |
| 45.3 | Male | African American | 21.5 | 6.73 | 8.1 | No | 68.9 | Non-Diabetes, Obesity | HYPERTENSIVE CARDIOVASCULAR DISEASE AND MORBID OBESITY |
| 52.6 | Female | African American | 26 | 6.38 | 9.1 | No | 36.9 | Non-Diabetes, Obesity | ACUTE FIBRINOUS PERICARDITIS WITH CARDIAC TAMPONADE |
| 66.7 | Female | Caucasian | 29.5 | 6.48 | 7 | No | 40.3 | Non-Diabetes, Obesity | RUPTURED THORACIC AORTIC ANEURYSM |
| 45.4 | Female | African American | 43 | 6.43 | 8.1 | No | 21.9 | Non-Diabetes, Normal | RUPTURED DISSECTING THORACIC AORTIC ANEURYSM |

**Supplemental Table 3**: Clinical data of human postmortem choroid plexus (PMI: postmortem interval; RIN: RNA integrity number; BMI: body mass index)

**Supplemental Table 3:** TaqMan probe and primer sequences of human *INS*, mouse *Ins2* isoforms, the autoantigen and insulin differentiation factor gene expression assay IDs (probe sequences are proprietary of Thermo Fisher Scientific).

| Isoforms | TaqMan probe | Forward primer | Reverse primer |
|---|---|---|---|
| Exon-2 | TGAACCAACACCTGTGCG | CCCAGCCGCAGCCTTT | AGAGAGCTTCCACCAGGTGTGA |
| INS3A | Hs00355773_m1 (NM_000207) | Exon-2 | Exon-3A (junction 126 bp amplicon) |
| EX2-I2 | ACGAGGCTTCTTCTACACAC | TCACACCTGGTGGAAGCTCTCT | GGCAGCAATGGGCAGTTG |
| INS1A | CCATCAAGCAGATCACTGT | GGACAGGCTGCATCAGAAGAG | ACAGGGCCATGGCAGAAG |
| INS1B | TTTGCGTCAGATCACT | CCATCAAGCAGGTCTGTTCCA | GGGCCATGGCAGAAGGA |
| INS1C | ACCCCAGATCACTGTC | CCATCAAGCAGGTCTGTTCCA | GCAGGAGGCGCATCCA |
| INS1I | CCATCAAGCAGGTCTG | GGACAGGCTGCATCAGAAGAG | AGCCCACCTGACGCAAAG |
| INSUA | CCATCAAGCAGATCACTGT | GGGAGATGGGCTCTGAGACTATAA | ACAGGGCCATGGCAGAAG |
| INSUB | TTTGCGTCAGATCACT | GGGAGATGGGCTCTGAGACTATAA | GGGCCATGGCAGAAGGA |
| INSUC | ACCCCAGATCACTGTC | GGGAGATGGGCTCTGAGACTATAA | GCAGGAGGCGCATCCA |
| INSU1 | CCATCAAGCAGGTCTG | GGGAGATGGGCTCTGAGACTATAA | AGCCCACCTGACGCAAAG |
| INS3B | CAGGCTGCCCTGCAG | TAGAGGGAGCAGATGCTGGTACA | AGGCTTCTTCTACACACCCAAGA |
| Ins2-V1 | CAAGCAGGAAGGTTATTGT | CCGCTACAATCAAAAACCATCA | CATCCACAGGGCCATGTTG |
| Ins2-V2 | CAAGCAGGAAGGTACTC | CCGCTACAATCAAAAACCATCA | GAGCCAGGCCCACTGAGA |
| Ins2-V3 | ATCCGCTACAATCAA | CCAGTAACCACCAGCCCTAAGT | AGATAGGCTTCCTGCTTGCTGATG |
| Ins2-V4 | CTGGAGGTTATTGTTTCAA | GCTCCTACGCTGAAATTCCAA | AAGGTGCTGCTTGACAAAAGC |
| IGF1 | Hs01547656_m1 (NM_000618) | exon-2 | exon-3 (junction 68 bp amplicon) |
| GAD2 | Hs00609534_m1 (NM_000818) | exon-15 | exon-16 (junction 95 bp amplicon) |
| PTPRN | Hs00160947_m1 (NM_001199763) | exon-3 | exon-4 (junction 65 bp amplicon) |
| SLC30A8 | Hs00545183_m1 (NM_001172811) | exon-5 | exon-6 (junction 73 bp amplicon) |
| ICA1 | Hs01119158_m1 (NM_001136020) | exon-13 | exon-14 (junction 77 bp amplicon) |
| MAFA | Hs01651425_s1 (NM_201589) | exon-1 | exon-1 (exon-1 121 bp amplicon) |
| NEUROD1 | Hs01922995_s1 (NM_002500) | exon-2 | exon-2 (exon-2 110 bp amplicon) |
| ISL1 | Hs00158126_m1 (NM_002202) | exon-5 | exon-6 (junction 57 bp amplicon) |
| NKX6-1 | Hs00232355_m1 (NM_006168) | exon-1 | exon-2 (junction 93 bp amplicon) |
| PDX1 | Hs00236830_m1 (NM_000209) | exon-1 | exon-2 (junction 73 bp amplicon) |
| PAX4 | Hs00173014_m1 (NM_006193) | exon-6 | exon-7 (junction 115 bp amplicon) |
| NEUROG3 | Hs01875204_s1 (NM_020999) | exon-2 | exon-2 (exon-2 117 bp amplicon) |

**Supplemental Table 4:** unlabeled and isotope-labeled (marked by asterisk) tryptic peptide sequences and their molecular weights (MW; dal, Dalton) for SRM-MS assay. CAM in the peptide sequences represents carbamidomethylation of cysteine residues to block its oxidation, and (^) the isotope labeled amino acid residues (hydrogen-2 for V and A, carbon-13 and nitrogen-15 for K, R and F).

| Peptide name | Peptide sequences | MW (dal) | AA |
|---|---|---|---|
| pep-B1 | $H_2N$-FVNQHLC[CAM]GSHLVEALYLVC[CAM]GER-OH | 2601.27 | 22 |
| pep-B2 | $H_2N$-GFFYTPK-OH | 859.43 | 7 |
| pep-A | $H_2N$-GIVEQC[CAM]C[CAM]TSIC[CAM]SLYQLENYC[CAM]N-OH | 2611.10 | 21 |
| pep-Cα | $H_2N$-EAEDLQGSLQPLALEGSLQ-OH | 1998.00 | 19 |
| pep-CαK | $H_2N$-EAEDLQGSLQPLALEGSLQK-OH | 2126.10 | 20 |
| pep-U1 | H2N-PAGAQQPSALQDR-OH | 1338.67 | 13 |
| pep-U2 | H2N-AFASGGLR-OH | 778.42 | 8 |
| pep-U3 | H2N-IPGWLDPR-OH | 953.52 | 8 |
| pep-U4 | $H_2N$-EDVAGLVK-OH | 830.46 | 8 |
| pep-U5 | $H_2N$-HVGVSPGAPR-OH | 976.53 | 10 |
| pep-US | H2N-AFASDHC[CAM]PSAMALWMR-OH | 1850.81 | 16 |
| pep-UF | $H_2N$-SHPAWAEGGR-OH | 1067.50 | 10 |
| pep-B1* | $H_2N$-FV^NQHLC[CAM]GSHLVEALYLVC[CAM]GER-OH | 2609.23 | 22 |
| pep-B2* | $H_2N$-GF^F^YTPK-OH | 867.00 | 7 |
| pep-A* | $H_2N$-GIV^EQC[CAM]C[CAM]TSIC[CAM]SLYQLENYC[CAM]N-OH | 2619.01 | 21 |
| pep-Cα* | H2N-EA^EDLQGSLQPLA^LEGSLQ-OH | 2004.00 | 19 |
| pep-CαK* | $H_2N$-EAEDLQGSLQPLALEGSLQK^-OH | 2134.10 | 20 |
| pep-U1* | H2N-PAGAQQPSALQDR^-OH | 1348.00 | 13 |
| pep-U2* | $H_2N$-AFASGGLR^-OH | 788.42 | 8 |
| pep-U3* | H2N-IPGWLDPR^-OH | 963.00 | 8 |
| pep-U4* | $H_2N$-EDVAGKVK^-OH | 838.46 | 8 |
| pep-U5* | $H_2N$-HVGVSPGAPR^-OH | 986.53 | 10 |
| pep-US* | H2N-A^FA^SDHC[CAM]PSA^MA^LWMR-OH | 1862.00 | 16 |
| pep-UF* | SHPA^WA^EGGR-OH | 1073.14 | 10 |

**Supplemental Table 5:** quantitation validation of SRM-MS assay in pooled postmortem cerebrum

| Target peptide | SRM | | | |
|---|---|---|---|---|
| | [a] Linear Regression and R^2 | [b] LOQ (nM) | CV (%) | [c] Accuracy (mean±SD, %) |
| Pep-B | $\log_2$ Con(nM) = 7.035+0.946 * $\log_2$Pep-B1(ratio, L/H); R^2 = .998 | 0.59 | 3.22 | 100.54±9.12 |
| Pep-A | $\log_2$ Con (nM) = 9.685+1.065 * $\log_2$Pep-A (ratio, L/H); R^2 = .998 | 4.69 | 7.03 | 101.11±6.40 |
| Pep-Cα | $\log_2$ Con(nM) = 7.51+1.04 * $\log_2$Pep-Cα (ratio, L/H)); R^2 = .997 | 6.25 | 3.43 | 98.28±8.76 |
| Pep-CαK | $\log_2$ Con(nM) = 7.32+0.97 * $\log_2$Pep-CαK (ratio, L/H); R^2 = .999 | 0.26 | 3.56 | 100.28±6.60 |
| Pep-U1 | $\log_2$ Con(nM) = 7.636+1.017 * $\log_2$Pep-U1 (ratio, L/H); R^2 = 1 | 0.38 | 2.04 | 100.23±5.53 |
| Pep-U3 | $\log_2$ Con(nM) = 6.926+1.031* $\log_2$Pep-U3 (ratio, L/H); R^2 = .999 | 3.32 | 0.81 | 100.26±7.43 |
| Pep-U4 | $\log_2$ Con(nM) = 10.655+1.02 * $\log_2$Pep-U4 (ratio, L/H); R^2 = .998 | 2.34 | 3.34 | 100.39±10.08 |
| Pep-UF | $\log_2$ Con(nM) = 7.519+0.991 * $\log_2$Pep-UF (ratio, L/H); R^2 = .999 | 0.21 | 5.95 | 100.40±8.57 |
| Pep-US | $\log_2$Con (nM) = 8.257 + .929 * $\log_2$Pep-US (ratio, L/H); R^2 = .998 | 7.81 | 2.87 | 100.30±8.57 |

[a] Linearity was determined by linear regression between measured concentration and peak area ratio (L/H) *versus* theoretical concentration determined by Light-SIS peptides (unlabeled form). All output of MultiQuant were weighted by $1/\chi^2$. Sum of all transition peak area ratio (L/H) to get peak area ratio at the peptide level.

[b] LOQ was determined from the standard curve, defined as the lowest limits of quantification calibrated with acceptable CV<20% and accuracy within 100±20%.

[c] Accuracy was estimated by back fitting data to the STD curve and average recovery from all quantified points in the plots. Data are mean±SD (%). Calibration curve is based on internal standard (IS unlabeled form) concentration and peak area ratio (transition ion peak area/IS peak area) and prepared in a pooled plasma matrix which was prepared from studying plasma samples >=5 points.

**Supplemental Table 6:** quantitation validation of SRM-MS assay in pooled human plasma

| Target peptide | SRM | | | |
|---|---|---|---|---|
| | [a] Linear Regression and R2 | [b] LOQ ng/ml | CV (%) | [c] Accuracy (%) |
| Pep-B | $log_2$Con(ng/ml) = 6.8 +0 .94 * $log_2$Pep-B1, ratio (L/H); R^2 = .998 | 1.52 | 3.77 | 100.36±9.74 |
| Pep-A | $log_2$Con(ng/ml) = 8.72 + 0.67 * $log_2$Pep-A, ratio (L/H); R^2 = .998 | 6.12 | 12.40 | 100.46±9.87 |
| Pep-CαK | $log_2$Con(ng/ml) = 7.118 + .942 * $log_2$Pep-CαK, ratio (L/H); R^2 = .999 | 3.32 | 3.20 | 100.15±5.46 |
| Pep-Cα | $log_2$Con(ng/ml) = 7.256 + .903 * $log_2$Pep-Cα, ratio (L/H); R^2 = .999 | 4.10 | 1.30 | 100.13±5.36 |
| Pep-U1 | $log_2$Con(ng/ml) = 5.926 + .988 * $log_2$Pep-U1, ratio (L/H); R^2 = 1 | 1.01 | 1.14 | 100.13±4.86 |
| Pep-U2 | $log_2$Con(ng/ml) = 4.43 + 1.046 * $log_2$ PepU2, ratio (L/H); R^2 = .998 | 0.20 | 1.56 | 103.25±11.40 |
| Pep-U3 | $log_2$Conng/ml = 4.039 + 1.023 * $log_2$ Pep-U3, ratio (L/H); R^2 = .998 | 0.84 | 1.15 | 100.42±9.48 |
| Pep-U4 | $log_2$Con(ng/ml) = 4.686 + 1.502 * $log_2$Pep-U4, ratio (L/H); R^2 = .998 | 0.12 | 1.23 | 98.92±12.57 |
| Pep-U5 | $log_2$Con(ng/ml) = 3.099 + 1.056 * $log_2$Pep-U5, ratio (L/H); R^2 = .998 | 0.57 | 1.79 | 100.69±12.22 |
| Pep-US | $log_2$Con, ng/ml = 6.87 + .944 * $log_2$Pep-US, ratio (L/H); R^2 = .999 | 14.46 | 2.04 | 114.21±6.07 |
| Pep-UF | $log_2$Con(ng/ml) = 5.732 + .958 * $log_2$Pep-UF, ratio (L/H); R^2 = .998 | 7.09 | 9.91 | 100.25±7.22 |

[a] Linearity was determined by linear regression between measured concentration and peak area ratio (L/H) *versus* theoretical concentration determined by Light-SIS peptides (unlabeled form). All output of MultiQuant were weighted by $1/\chi^2$. Sum of all transition peak area ratio (L/H) to get peak area ratio at the peptide level.

[b] LOQ was determined from the standard curve, defined as the lowest limits of quantification calibrated with acceptable CV<20% and accuracy within 100±20%.

[c] Accuracy was estimated by back fitting data to the STD curve and average recovery from all quantified points in the plots. Data are mean±SD (%). Calibration curve is based on internal standard (IS unlabeled form) concentration and peak area ratio (transition ion peak area/IS peak area) and prepared in a pooled plasma matrix which was prepared from studying plasma samples >=5 points.

**Sample preparation and SRM-MS analysis**

Under a stereo microscope, approximately 200 intact islets from individual donors were handpicked into a polypropylene tube containing 1 ml of ice-cold PBS. After washing twice with 1 ml PBS containing 1X protease inhibitor cocktails, the islets were resuspended in 100 µl of 0.1% RapiGest (w/v) (Waters Corp., Milford, MA) containing 100 mM Tris-HCl (pH 8.0) and 100 nM DTT. For choroid plexus postmortem frozen sections 100 µl of 0.1% RapiGest was added on each slide, and then tissue was scraped off into a clean 1.5 ml of tube. Then, islet and choroid plexus in 0.1% RapiGest were sonicated 3 x 3 seconds on/30 seconds off on ice. After centrifugation (16,000g, 20 min at 4°C), supernatants were collected and protein concentration was determined by BCA assay (Cat#: 23225, Thermo Fisher Scientific, Waltham, MA), and stored at -80°C until further

analysis. Due to very low amounts of islet lysate, the islet protein concentration was not determined, and thus relative quantification was used for quantification, *e.g.,* the ratio of pep-CαK/pep-Cα and pep-Us/pep-B2.

Fresh-frozen human plasma was thawed on the day of analysis. After centrifugation (16,000g, 15 min at 4°C), cleared fractions were transferred with a loading tip into a fresh 1.5 mL polypropylene tube, discarding insoluble aggregates and the upper layer of floating lipids. This procedure was enough to eliminate the confounding influence of lipids on downstream protein separation procedures. Then 5 µl of delipidated plasma was mixed with 95 µl of 0.1% RapiGest.

Tryptic digestion was performed with an automated robotic procedure aimed at minimizing sample handling variability in a flow for SRM analysis(1). Briefly, sample lysate (100 µl) in 0.1% RapiGest were transferred into the reaction plate, incubated 1 hour at 55°C for denaturation and reduction, followed by 30 min alkylation with a fresh made 0.1 M solution of iodoacetamide (Sigma-Aldrich) to a final concentration of 50 mM at room temperature in the dark. After alkylation, trypsin/LysC mix (Promega, Madison, WI) was added at an enzyme-to-substrate ratio of 1:50. Digestion was carried out for 18 hours at 37°C and terminated with 10% MS-grade trifluoroacetic acid (Fisher Scientific, Hampton, NH) to a final concentration of 1%. Acidified tryptic digests were cleaned up with 96-well SPE plate (Phenomenex, Torrance, CA) according to manufacturer's instruction. A 96-well plate vacuum manifold (Waters Corp., Milford, MA) was used for all desalting procedures to provide uniform peptide wash, retention, and elution. The elution reagents were evaporated to dryness and stored at -80°C until SRM analysis. All internal standard peptides of the novel *INS* uORF isoforms (INSU1 and INSU2) were post-spiked into tryptic digests. the tryptic pep-U3 is measured and validated by SRM-MS because the arginine digestion site (0) is flanked by prolines at -1 (promoting) and +1 (inhibiting) sites (2; 3).

We used a Shimadzu LC-HPLC equipped with LC-20ADXR pumps (Shimadzu Corp., Columbia, MD) for solvent and sample delivery and a 2.1 mm X 100 mm, 130 Å pore size, 3.5 µm particle size C18 column (Waters Corp.) for the peptide separations using the following linear gradient: 0min 5%B; 10 min 36%B; 12 min 90%B; 13.5 min 90%B; 14 min 5%B at a flow rate of 0.2ml/min. The total run time was 18 min per sample. Triplicate injections of 10 µl of sample were carried out via the SIL-20AXR autosampler (Shimadzu Corp.). To eliminate possible carryover, the column was re-equilibrated at 50%B for 10 min and a blank run was performed prior to initiating the next sample injection. QTRAP 5500 mass spectrometer with electrospray ionization (ESI) source controlled by Analyst 1.6.3 software (AB Sciex, Framingham, MA) was used for all LC-MS/MS detection and analysis. Mass spectrometric analyses were performed in positive ion mode. ESI interface parameters were set as follows: capillary temperature 650°C and a curtain gas setting of 30 psi. By using scheduled SRM, a total of 148 SRM transitions from 15 peptides were monitored during an individual sample analysis with Q1 and Q3 set by declustering potential (DE) 10 V and peptide-specific tuned collision energy (CE), entrance potential (EP) and collision cell exit (CXP) voltages for each transition.

1. Zhu M, Zhang P, Geng-Spyropoulos M, Moaddel R, Semba RD, Ferrucci L: A robotic protocol for high-throughput processing of samples for selected reaction monitoring assays. Proteomics 2017;17
2. Eisenhaber F, Eisenhaber B, Kubina W, Maurer-Stroh S, Neuberger G, Schneider G, Wildpaner M: Prediction of lipid posttranslational modifications and localization signals from protein sequences: big-Pi, NMT and PTS1. Nucleic Acids Res 2003;31:3631-3634
3. Pan Y, Cheng K, Mao J, Liu F, Liu J, Ye M, Zou H: Quantitative proteomics reveals the kinetics of trypsin-catalyzed protein digestion. Anal Bioanal Chem 2014;406:6247-6256