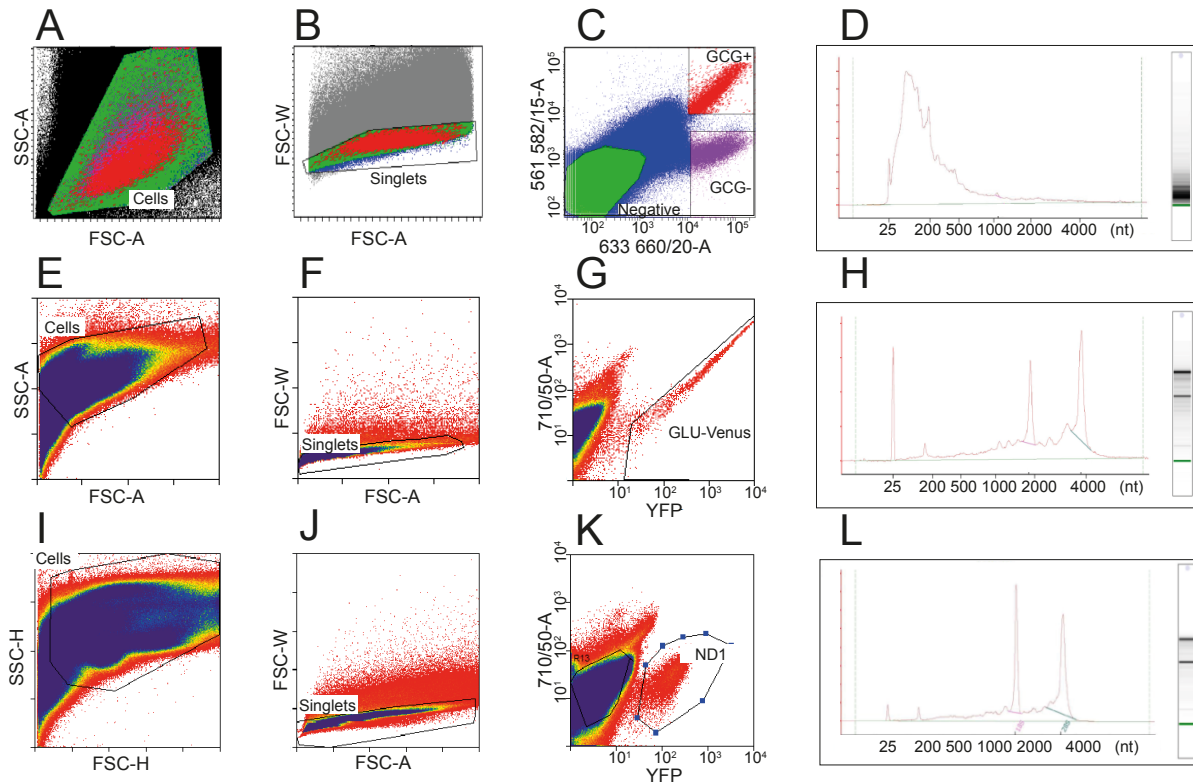
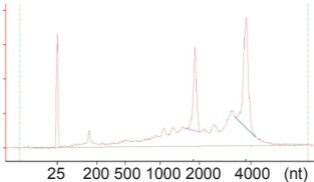


SUPPLEMENTARY DATA

Supplementary Figure 1. (A,B,C) FACS sorting of human GCG+, GCG- and negative cells. Cells were sorted by side scatter (SSC), forward scatter (FSC-A) and pulse width (FSC-W) to collect single cells. CHGA/SCG2 staining was detected by 633nm excitation, 660/20nm emission; GLP-1 staining was detected by 561nm excitation, 582/15nm emission. (E,F,G) FACS sort of murine GLU-Venus cells using gating as labelled. (I,J,K) FACS sort of murine NeuroD1-Cre/ROSA26-YFP cells using gating as labelled. (D,H,L) Agilent Bioanalyzer 2100 trace of RNA quality from human GCG+ (D) and murine GLU-Venus (H) and NeuroD1 (L) cell populations.

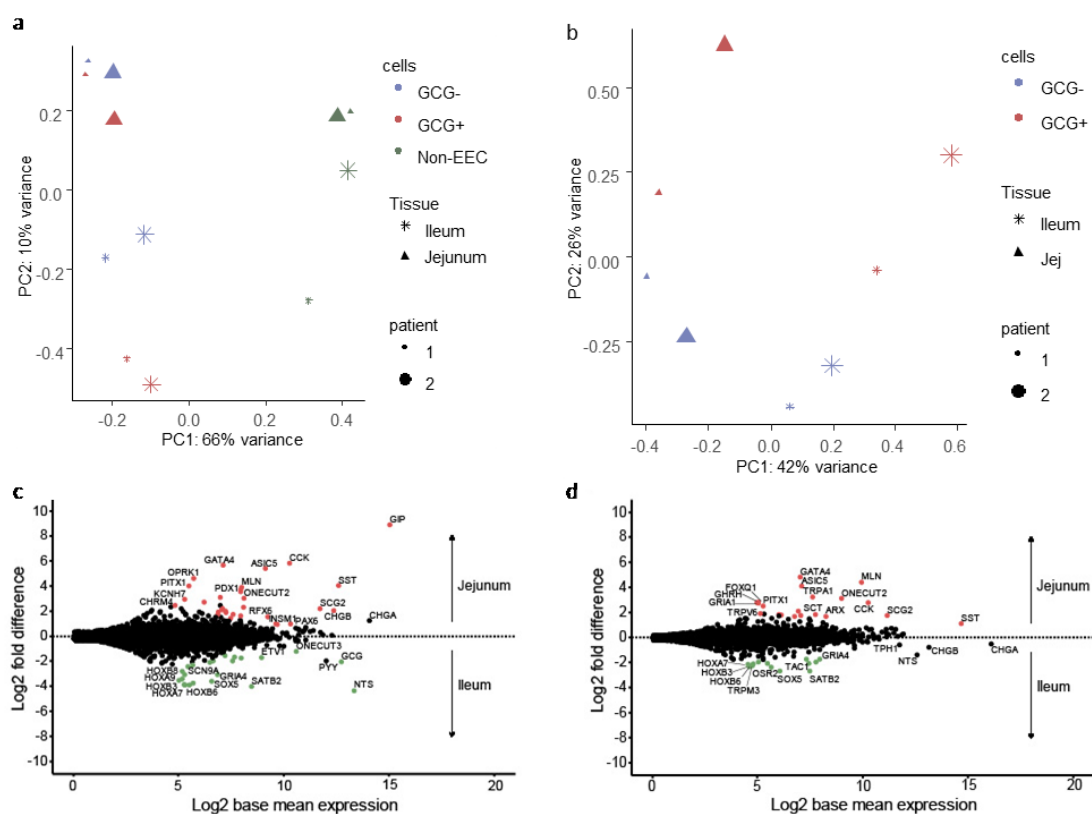


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Supplementary Figure 2. Comparison between EECs from human jejunum and ileum



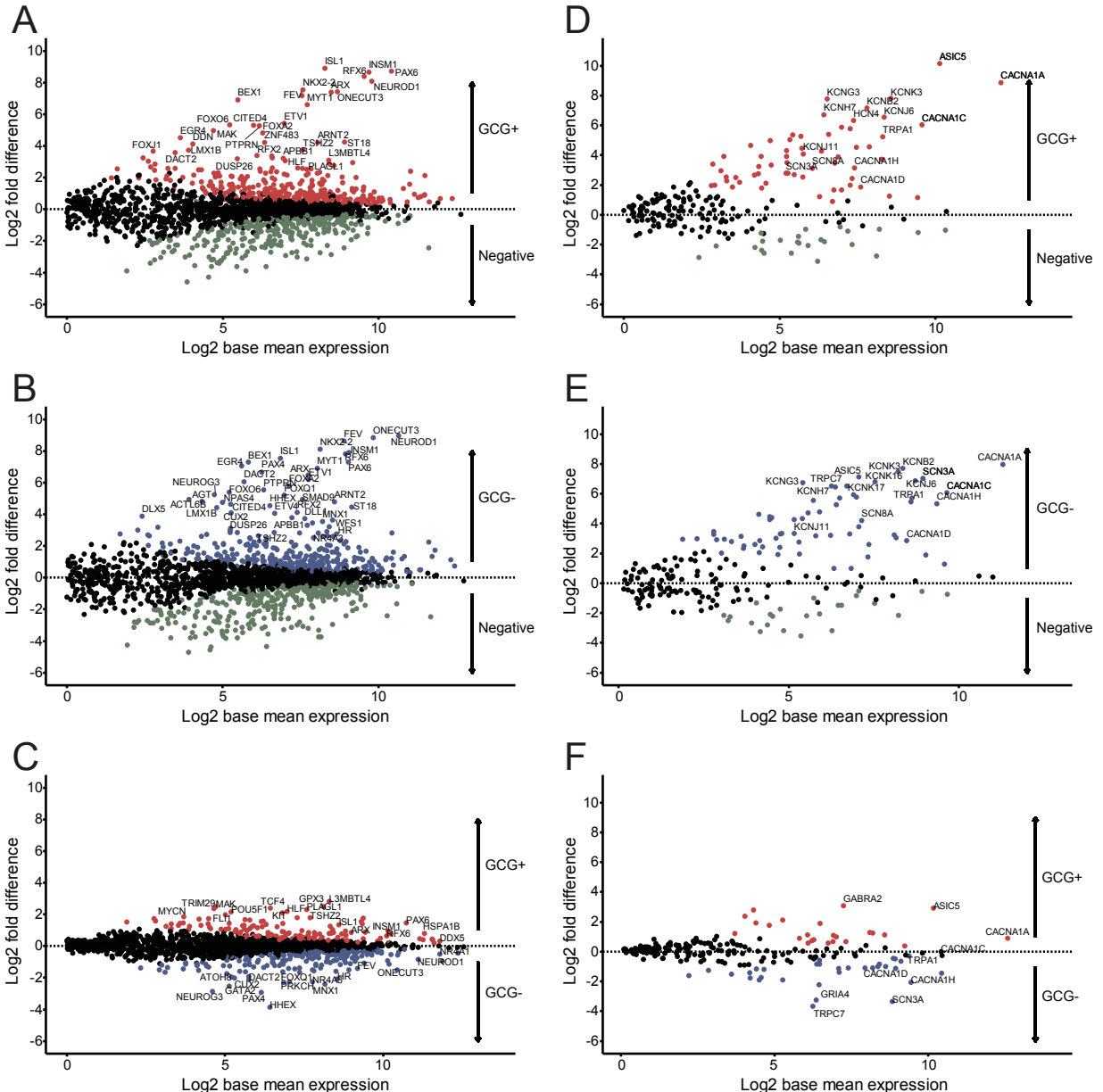
(a) Principal component analysis (PCA) plot of matched human jejunum vs ileum samples, labelled by cell population, anatomical location and patient ($n=3$ cell populations from both anatomical regions from each of 2 participants) of the 500 top variable genes when using a DESEQ2 model. PCA separated EECs from negative cells on the first component and the anatomical location of GCG+ and GCG- cells on the second component. (b) PCA plot for the same samples as in (a), but excluding the non-enteroendocrine (Neg) samples from the DESEQ2 model and the PCA analysis. PCA showed separation of ileal from jejunal samples on the first component and GCG+ from GCG- on the second component. Samples from the different participants are depicted by different sized symbols.

(c,d) Enrichment vs expression plots for GCG+ (c), and GCG- (d), cell populations. Enrichment is presented as the log₂ fold difference between the cell populations indicated, and expression is presented as the log₂ base mean normalised expression extracted from the DESEQ2 model. Red – enriched (adjusted $P \leq .1$ in DESEQ2 model) in jejunum; Green – enriched in ileum.

GIP, *CCK*, *SST*, *MLN*, *SCT*, *GHRH*, *ASIC5*, and *TRPA1* were notably higher in jejunal than ileal EECs (GCG+ or GCG-), whereas *GCG*, *NTS* and *TAC1* were higher in ileum than jejunum.

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Supplementary Figure 3. Differential vs mean expression plots for human jejunum EEC populations. Differential expression is presented as the \log_2 fold difference between the cell populations indicated, and expression is presented as the \log_2 base mean normalised expression extracted from the DESEQ2 model. (A,D) GCG+ vs negative, (B,E) GCG- vs negative (C,F), GCG+ vs GCG-. Transcription factors are shown in A,B,C; ion channels are shown in D,E,F. Red – enriched (i.e. adjusted $P < 0.1$) in GCG+; Blue – enriched in GCG-; Green – enriched in negative cells. Key genes are labelled



SUPPLEMENTARY DATA

Supplementary Figure 4 . Peptidomics optimisation studies

Peptidomics is a relatively new and under-developed field within proteomics, despite the varied and significant roles of peptides as signalling molecules in many organisms. Peptides, due to their small size in comparison to proteins, have specific properties that can be used to extract and analyse them intact by mass-spectrometry, giving information about their exact processing and post-translational modifications. Several methods to extract peptides from different tissues or fluids have been published, but none was developed to study intestinal tissues and the large variety of hormone peptides they produce.

Peptide extraction and purification for LC-MS/MS studies initially requires the complete disruption of the tissue of interest while inhibiting all protease activity. This is to avoid contamination with degradation products of larger proteins as well as degradation of the peptides of interest. The low-molecular weight proteins and peptides must then be separated from the rest of the proteins and other molecules. We compared several methods adapted from the literature to develop a robust and reliable method to quantify peptide levels from intestinal tissues. Methods tested for tissue lysis and protein extraction included tissue homogenization in 0.25% acetic acid, 8M urea, 90% methanol with 1% acetic acid, guanidine hydrochloride or 80% acetonitrile, or using Trizol to extract both proteins and peptides from the same sample¹⁻⁴ Peptides were separated from proteins using either 10kDa molecular weight cut-off filters or 80% acetonitrile. Samples were finally purified using SPE columns, dried and reduced alkylated before being analysed by mass-spectrometry.

The different methods allowed the detection of many unique peptides by LC-MS/MS with an important variability between the different methods, from 1778 unique peptides for the extraction using 8M urea to 25 with 90% methanol/1% acetic acid. Many corresponded to fragments of longer peptides or proteins and could represent degradation products, coming either from the protein turnover of the tissue or degradation happening during sample preparation. However, most of the peptides were unique to the extraction method (SupFig 4a), due to their different chemical properties and also due to different degradation levels. Focusing on the analysis of the classical gut peptide hormones significantly reduced the number of hits, and clearly showed that most of the expected intact peptides were detected using the guanidine HCl extraction method (SupFig 4b). In addition to improving the number of detected hormone peptides, the guanidine HCl extraction method showed the highest levels of these peptides when quantified (SupFig 4c). Urea and Trizol extraction were the second best methods for peptide levels and variety of peptide hormones extracted, and tissue homogenization using Trizol can be a reliable technique to extract both RNA and peptides from a same sample. We also compared peptide extraction from the proteins using either protein precipitation with 80% ACN or using 10kDa filters, the latter resulting in the loss of many peptides and decreased levels (Supp Fig 4c).

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We then confirmed the reproducibility of the guanidine HCl +80% ACN extraction method, as the %CV from 5 replicates repeated twice from different mice was around 20% for most of the peptides, with higher %CV for peptides detected with lower abundance (Supp Fig 4d), and we could also show that peptide quantification was linear with the amount of starting material in a range from 2 to 40 mg (Supp Fig 4e), indicating that tissue weight can be a satisfactory normalisation factor to compare different samples.

Tissue homogenization in guanidine HCl followed by protein precipitation with 80% acetonitrile is therefore a promising method to extract peptides from intestinal tissue for mass-spectrometry analysis, allowing the detection a large range of peptides and their robust quantification.

Homogenization optimization

Small pieces of mouse jejunal mucosa of similar size (~40mg) were homogenized in different conditions in duplicates with lysing Matrix D using a FastPrep-24 for 4 x 40s at 6ms⁻¹, and peptide extracted before being dried on a centrifugal concentrator. Samples were resuspended in 500µL 0.1% aqueous formic acid and purified by solid-phase extraction using a Waters HLB µElution solid-phase extraction (SPE) plate as described. Samples were analysed using high-flow rate based LC-MS method as described before.

For the acetic acid, guanidine HCl and urea extractions, tissues were directly incubated in 300µL of the lysing solution (0.25% acetic acid aqueous, guanidine HCl 6M or urea 8M) and homogenized. 200µL of the homogenate was collected into a new tube after centrifugation to remove tissue debris and lysing matrix (5min, 2000g, 4°C). 80% acetonitrile (800µL) was added to precipitate the proteins and peptides in solution were collected following centrifugation (10min, 12,000g, 4°C). For the guanidine HCl homogenates, addition of 80% acetonitrile induced a separation between an organic phase (top) and aqueous phase (bottom) in addition to the protein precipitation, with the peptides in the aqueous phase (data not shown). Peptides from guanidine HCl homogenization were also extracted using a Vivaspin 500 MCWO 10kDa (GE-healthcare) instead of using 80% ACN, collecting the flow-through after a 30 minutes spin at 16,000g at 4°C. Flow-through was then acidified adding 10% formic acid solution and extracted by SPE.

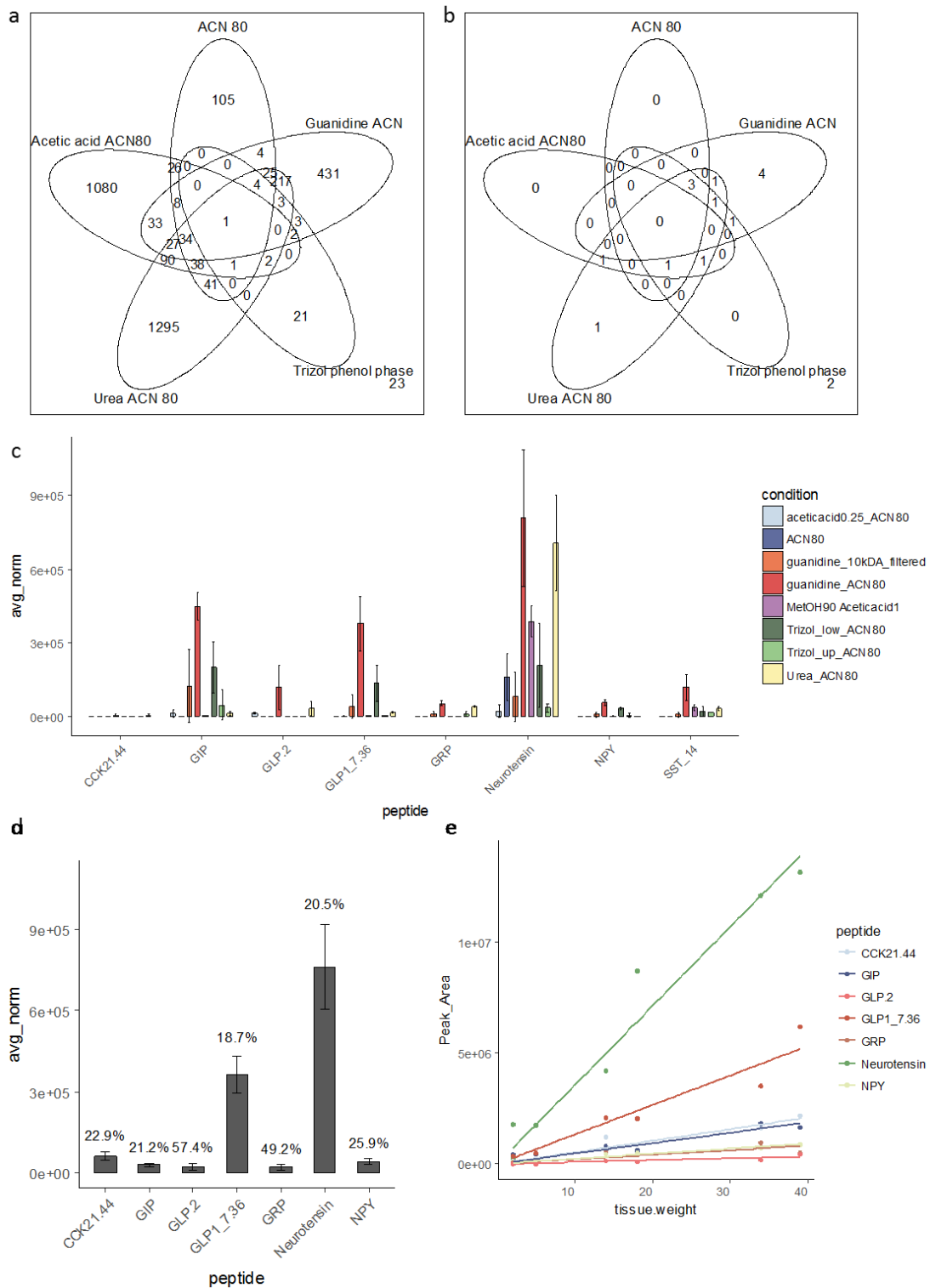
For the 80% ACN and methanol/acetic acid extraction, tissues were directly homogenized in 80% acetonitrile aqueous or 90% methanol 1% aqueous acetic acid, 200 µL of homogenate was recovered from the lysing D matrix and spun at 12,000g at 4°C for 10 minutes to separate the precipitated proteins. Peptides in supernatant were transferred to a new tube and dried before SPE extraction as previously.

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For the Trizol extraction, tissue was homogenized in 500 μ L Trizol and the homogenates transferred to a new tube. 100 μ L chloroform was added and mixed vigorously and sample centrifuged after 10min incubation at RT (15min, 12,000g, 4°C). The upper phase (aqueous) was then transferred to a new tube (from which RNA can be extracted using standard methods), 800 μ L 80% ACN was added to precipitate any remaining proteins and sample centrifuged (10min, 12,000g, 4°C) and supernatant dried before SPE extraction. The lower (phenol) phase was treated with 100% ethanol (1:3 ethanol:phenol phase by volume) to precipitate DNA and the mixture centrifuged (10min, 2,000g, 4°C) then transferred to a new tube. Proteins and peptides were precipitated by addition of 750 μ L cold acetone and centrifugation at 7500g for 5min at 4°C. Pellets were washed once with 1mL 0.3M guanidine HCl in ethanol and air dried before being resuspended in 200 μ L 8M urea. Finally, proteins were precipitated with 80% ACN, centrifuged (10min, 12,000g, 4°C) and the supernatant (containing the peptides) was transferred to a fresh tube, dried and SPE extracted as previously.

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2. Van Camp, K. A., Baggerman, G., Blust, R. & Husson, S. J. Peptidomics of the zebrafish *Danio rerio*: In search for neuropeptides. *J. Proteomics* **150**, 290–296 (2017).
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4. Ziros, P. G., Chartoumpekis, D. V. & Sykiotis, G. P. A Simple Protocol for High Efficiency Protein Isolation After RNA Isolation from Mouse Thyroid and Other Very Small Tissue Samples. in 383–393 (Humana Press, New York, NY, 2016). doi:10.1007/978-1-4939-3756-1_25

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a and b: Venn diagrams showing the total number of unique peptides (a) and the number of unique peptides matching classical hormone peptides (b) found in the different methods for peptide extraction. c: Quantification of different gut hormone peptide using different extraction methods, normalised by tissue weight. Data is represented as mean +sd from 2 replicates. d: Quantification variability for different peptides measured from 10 homogenates from 2 different mice. Data is the mean value +sd and values indicated above are the %CV. e: Raw peak area of peptide quantification for different samples spanning different weights from 2 to 40 mg plotted against the tissue weight. Linear regression is plotted, showing a good correlation between tissue weight and peptide quantification.

SUPPLEMENTARY DATA

Supplementary Table 1. Demographics of human participants from transcriptomic study. RYGB = Roux-en-Y Gastric Bypass; donor = organ transplant donor; TG = Total gastrectomy for prevention / treatment of gastric cancer

ID	Source	Age	Gender	BMI
R3	France – RYGB	N/A	N/A	>40
R4	France – RYGB	24	F	47
R5	France – RYGB	31	F	43
R6	France – RYGB	46	M	41
D1	France – donor	27	M	24
D2	France – donor	26	F	21
HGH006	UK – TG	57	M	34
HGH007	UK – TG	22	M	29
HGH008	UK – TG	23	M	28
HGH010	UK – TG	64	M	27
HGH018	UK – TG	61	M	37