**Supplementary Material**

**Pharmacokinetics and In Vivo Efficacy of Optimized Apidaecin Derivatives**

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Method M1: Multiple reaction monitoring (MRM) transitions for apidaecin peptides

Peptides (1 µg/mL) were dissolved in aqueous acetonitrile (25% or 60% v/v) containing either formate buffer (26 mmol/L, pH 2.6, 3.0, 3.3 or 3.9) or formic acid (0.1 % v/v) and infused (flow rate 5 µL/min, PHD 2600 syringe pump) into the ESI-QqLIT-MS (4000 QTRAP®) to optimize buffer composition and MRM. Transitions were selected from the tandem mass spectra (MS/MS) and confirmed on an ESI-QqTOF-MS (QSTAR® pulsar I) providing higher mass resolutions and mass accuracies than the QTRAP®. The settings for declustering potential (DP), collision potential (CE), and collision cell exit potential (CXP) were optimized by ramping the respective potentials using the compound optimization tool of the Analyst® software.

Method M2: Sample preparation

Frozen organs (5 per group) were homogenized in sterile PBS (500 µL) containing Protease Inhibitor Mix M (1% v/v; SERVA Electrophoresis GmbH, Heidelberg, Germany) using an Ultra-Turrax® T25 basic (IKA-Werke, Staufen, Germany, rotational speed 21,500 min⁻¹) for 1 min. Homogenates were sonicated on ice (35 % amplitude, mode: 5 s on; 5 s off for 1 min; Vibra-cell™ 75041, Thermo Fisher Scientific) to disrupt cells. After centrifugation (15 min, 21,460 x g, 4°C) the supernatants were stored at -80°C.

Aliquots of plasma (10 µL) or liver (10 µL), kidney (50 µL), and brain homogenates (100 µL) were spiked with isotope-labeled Api88, Api137, Api1-16, and Api1-17 (2.5 µg/mL). The samples were diluted fourfold with aqueous phosphoric acid (3.4 % v/v), loaded on an Oasis HLB 96-well solid phase extraction (SPE) plate (5 mg sorbent, Waters), washed with aqueous methanol (0.3 mL, 5% v/v), eluted with methanol (0.3 mL), and dried in a vacuum centrifuge (30 °C; SpeedVac, Eppendorf). The samples were dissolved prior analysis in aqueous acetonitrile (3% v/v) containing formic acid (0.1 % v/v).

Stock solutions (1 g/L) of the peptides and their corresponding isotope-labelled internal standards were prepared and diluted in water. Calibration and validation mixtures (10 µL) of unlabeled (0.025, 0.1, 0.5 or 2.5 µg/mL) and isotope-labeled peptides (2.5 µg/mL) were mixed with plasma or organ homogenates (10 to 100 µL) obtained from untreated animals for calibration.

Limits of detection (LOD) and quantification (LOQ) corresponded to the lowest peptide concentrations in water, plasma, or homogenates of brain, liver, and kidney providing a peak height exceeding the noise in the retention time window at least threefold (LOD) or ten times (LOQ). Recovery rates of SPE and matrix effects were determined by the peak areas of spiked
plasma samples and organ homogenates relative to equally concentrated peptide solutions in pure water. Concentrations of samples below the LOQ but still detectable were set to the value of the LOD, whereas signals with peak heights below the LOD were set to 0 µg/mL.

**Method M3: Recovery rates in plasma and organ homogenates**

Li-heparin blood samples obtained from untreated NMRI mice were spiked with Api88 or Api137 (final concentrations: 2.5 or 10 µg/mL), incubated (10 min, RT), and centrifuged (2,000x g, 3 min, RT) to obtain plasma. Frozen organs were homogenized with PBS (0.5 mL) containing protease inhibitor mix M (SERVA; 1 % v/v) and either Api88 (brain: 500 µL, 0.2 or 1 µg/mL, liver: 500 µL, 2 or 5 µg/mL, kidneys: 500 µL, 20 or 60 µg/mL) or Api137 (brain: 500 µL, 0.2 or 2 µg/mL, liver: 500 µL, 1 or 2.5 µg/mL, kidneys: 500 µL, 20 or 60 µg/mL). The analysis followed the protocol described above for pharmacokinetics samples. Recoveries were calculated by assuming a total blood (plasma) volume of 75 (25) mL per kg mouse weight and that the measured organ weight (in g) corresponds to the organ volume (in mL).

**Method development and validation**

Apidaecin peptides were selectively and sensitively quantified based on the methods established for oncocins Onc72 and Onc112 (Schmidt R. et al JAC 2016). MRM based on triply charged precursor ions (Figure S1A and C) and a neutral loss of two dimethyl amine molecules cleaved from the N-terminal guanidine group as most intense fragment ions (Figure S1B and D). To avoid misinterpretation of possible cross talks, an additional MRM transition with an m/z above the m/z selected in quadrupole 1 was used (Table S4). MS settings were optimized in order to obtain the highest sensitivities (Table S4) and the pH of the eluents was elevated to 3.0 to increase the intensity of the signals corresponding to the triply protonated precursor ions (Figure S2). Interfering sample components, such as proteins, salts, and lipids, were removed by solid phase extraction (OASIS HLB plates) before the samples were analyzed by RP-HPLC-ESI-QqLIT-MS (flow rate: 0.2 mL/min, column temperature: 55°C, linear gradient from 2.7 to 20.7% acetonitrile containing 26 mmol/L formate buffer (pH 3.0) in five minutes) with an overall duration of 20 min. Polypropylene tubes were used for all handling steps, as the highly basic peptides adsorbed strongly to glass surfaces resulting in a hundred fold reduced sensitivity.

The method provided instrumental LODs of 14 and 28 ng/mL for Api88 and Api137, respectively, and only slightly higher LOQs (Table S6). The linear dynamic range spanned
more than two orders of magnitude up to 10 µg/mL. When all steps of the analytics were considered, the LODs increased for Api137 and even more for Api88 (Table S9), most likely through losses during SPE, which had recoveries between 35 and 56% for all four peptides, with higher recoveries for low concentrations (Table S7). The successful removal of isobaric components was confirmed by the absence of signals after injection of plasma (untreated animals) after SPE. The weak matrix effects on the signal intensities (<10%) with enhancing effects at low peptide concentrations further confirmed this. The precisions and accuracies met the requirements very well (Table S8) indicating that the developed method allowed a reliable and sensitive quantification of apidaecin peptides in biological samples. However, the LODs and LOQs increased in organ homogenates, especially for liver, and in urine samples (Table S9).
Table S1. Sequences, monoisotopic masses, transitions of MRM quantification, and analytical parameters of all apidaecin peptides analyzed in murine plasma samples.

| Peptide* | Sequence* | [M+H]$^+$ | MRM mass ranges |
|----------|-----------|------------|-----------------|
|          |           | Theor.     | Exp.            | Q1               | Q3               |
| Api88    | gu-ONNRPVYIPPRPPHPRL-NH$_2$ | 2290.36 | 2290.42 | 764.13 | [M+3H]$^{3+}$ | 734.09 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Api88 IS | gu-ONNRPVYIPPRPPHPRL-NH$_2$ | 2296.36 | 2296.47 | 766.13 | [M+3H]$^{3+}$ | 736.09 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Api137   | gu-ONNRPVYIPPRPPHPRL-OH | 2291.35 | 2291.41 | 764.45 | [M+3H]$^{3+}$ | 734.41 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Api137 IS| gu-ONNRPVYIPPRPPHPRL-OH | 2297.35 | 2296.49 | 766.45 | [M+3H]$^{3+}$ | 736.41 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Metabolite 1-17 | gu-ONNRPVYIPPRPPHP-PR-OH | 2178.26 | 2178.30 | 726.80 | [M+3H]$^{3+}$ | 696.70 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Metabolite 1-17 IS | gu-ONNRPVYIPPRPPHP-PR-OH | 2184.26 | 2184.31 | 728.80 | [M+3H]$^{3+}$ | 698.70 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Metabolite 1-16 | gu-ONNRPVYIPPRPPHP-OH | 2022.16 | 2022.24 | 674.70 | [M+3H]$^{3+}$ | 644.70 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |
| Metabolite 1-16 IS | gu-ONNRPVYIPPRPPHP-OH | 2028.16 | 2028.22 | 676.70 | [M+3H]$^{3+}$ | 646.70 | [MH$_3$-2 NHMe$_2$]$^{3+}$ |

* IS denotes isotope-labeled peptides

# gu denotes $N,N,N',N''$-tetramethylguanidine, $[^{13}$C$_5,^{15}$N]-L-proline residues are underlined
Table S2. Equipment used for RP-HPLC-ESI-MS.

| Company                                      | Instrument/software                               |
|----------------------------------------------|--------------------------------------------------|
| ABSciex Germany GmbH, Darmstadt, Germany     | Analyst® 1.6 software                             |
|                                              | ESI-QqLIT-MS (4000 QTRAP®)                        |
|                                              | ESI-QqTOF-MS (QSTAR® pulsar I)                    |
| Harvard Apparatus INC., Holliston, MA, USA   | PHD 2600 syringe pump                             |
| Phenomenex® Ltd. Torrance, USA              | Jupiter C18-column                                |
|                                              | (1 mm internal diameter, 150 mm length, 5 µm     |
|                                              | particle size, 30 nm pore size)                   |
| Thermo Fisher Scientific GmbH, Dreieich,     | ESI-LTQ-Orbitrap XL™-MS                           |
| Germany                                      | Xcalibur™ software                                |
| Waters GmbH, Eschborn, Germany              | nanoACQUITY UPLC®                                |
|                                              | BEH C18 nanoACQUITY UPLC® column                 |
|                                              | (75 µm internal diameter, 100 mm length, 1.7 µm  |
|                                              | particle size, 13 nm pore size)                   |
|                                              | MassLynx™ software                                |
|                                              | Progenesis® QI software                           |
|                                              | Alliance® 2695 HPLC system                        |
Table S3. Turbo V™ ion source and mass analyzer settings of the ESI-QqLIT-MS (4000 QTRAP®) for analyzing Api88, Api137, Api1-16, and Api1-17.

| Parameter                          | Turbo V™ ion source settings                                                                 |
|------------------------------------|------------------------------------------------------------------------------------------------|
| Sample introduction                | Alliance® 2695 HPLC System                                                                  |
| Scan type                          | Multiple reaction monitoring                                                                |
| Sample introduction flow rate      | 200 µL/min                                                                                  | 5 µL/min                                      |
| Ion spray voltage (IS)             | 5500 V                                                                                      | 5500 V                                        |
| Nebulizing gas (Gas 1)             | 50 psi                                                                                      | 10 psi                                        |
| Drying gas (Gas 2)                 | 50 psi                                                                                      | -                                              |
| Curtain gas (CUR)                  | 30 psi                                                                                      | 15 psi                                        |
| Temperature (TEM)                  | 650 °C                                                                                      | -                                              |
| Interface heater                   | on                                                                                          | on                                             |

Mass analyzer settings

| Quadrupole (Q1) resolution         | Unit                                                                                      | Low (for product ion scan)                   |
| Quadrupole (Q3) resolution         | Unit                                                                                      | -                                              |
| Collision gas (CAD gas)            | High                                                                                      | High (for product ion scan)                  |
Table S4. MS/MS settings of the 4000 QTRAP® to quantify Api88, Api137, Api1-16, and Api1-17 as well as the corresponding isotope-labeled standard peptides.

| Peptide   | Type       | Precursor     | Fragment      | DP_{opt} [V] | CE_{opt} [V] | CXP_{opt} [V] |
|-----------|------------|---------------|---------------|--------------|--------------|--------------|
| Api88     | Quantifier | [M+3H]^3     | [MH3-2 NHMe2]^3+ | 764.13       | 734.09       | 83           |
|           | Qualifier  | [M+3H]^3     | y^{3}_{16}–NH_{3} | 1031.09      | 83           | 43           |
|           | IS         | [M+3H]^3     | [MH3-2 NHMe2]^3+ | 766.13       | 736.09       | 83           |
| Api137    | Quantifier | [M+3H]^3     | [MH3-2 NHMe2]^3+ | 764.45       | 734.41       | 58           |
|           | Qualifier  | [M+3H]^3     | y^{2}_{16}     | 1040.10      | 58           | 39           |
|           | IS         | [M+3H]^3     | [MH3-2 NHMe2]^3+ | 766.45       | 736.41       | 58           |
| Metabolite 1-17 | Quantifier | [M+3H]^3   | [MH3-2 NHMe2]^3+ | 726.80       | 696.70       | 58           |
|           | Qualifier  | [M+3H]^3   | [MH3-NHMe2]^3+  | 711.70       | 58           | 35           |
|           | IS         | [M+3H]^3   | [MH3-2 NHMe2]^3+ | 728.80       | 698.70       | 58           |
| Metabolite 1-16 | Quantifier | [M+3H]^3   | [MH3-2 NHMe2]^3+ | 674.70       | 644.70       | 58           |
|           | Qualifier  | [M+3H]^3   | y^{3}_{16}–NH_{3} | 598.30       | 58           | 39           |
|           | IS         | [M+3H]^3   | [MH3-2 NHMe2]^3+ | 676.70       | 646.70       | 58           |

DP, CE, and CXP denote declustering, collision, and collision cell exit potentials, respectively.
Table S5. Ion source and mass analyzer settings applied on the ESI-LTQ-Orbitrap XL™-MS to identify metabolites of Api88 and Api137 in plasma samples.

| Parameter                        | Setting       |
|----------------------------------|---------------|
| Ionization mode                  | Positive      |
| Resolution                       | 60000         |
| Ion spray voltage (IS)           | 1500 V        |
| Aux gas flow rate                | 1 arb         |
| Capillary temperature            | 200 °C        |
| Tube lens voltage                | 120 V         |
| Mass-to-charge ratio (m/z) range | 200 – 2000    |

Table S6. Analytical parameters calculated from a serial dilutions series of Api88 and Api137 analyzed by the optimized MRM method. Peptide solutions (10 µL) were prepared in water without any biological matrix.

| Peptide | LOD   | LOQ   | LDR   | R²   |
|---------|-------|-------|-------|------|
| Api88   | 14 ng/mL | 15 ng/mL | 7*10² | 0.9911 |
| Api137  | 28 ng/mL | 29 ng/mL | 3*10² | 0.9988 |

LOD, LOQ, and LDR denote limit of detection, limit of quantification, and linear dynamic range, respectively.
Table S7. Recovery rates and matrix effects after SPE preparation of peptides spiked in murine heparin plasma and analyzed with the optimized MRM method.

| Peptide | Concentration [ng/mL] | Recovery after SPE [%] | Matrix effects [%] |
|---------|------------------------|------------------------|-------------------|
| Api88   | 100                    | 56 ± 16                | 13 ± 3            |
|         | 600                    | 50 ± 15                | -1.9 ± 0.3        |
|         | 5000                   | 47 ± 11                | -4.7 ± 0.5        |
| Api137  | 100                    | 45 ± 2                 | 3.3 ± 0.2         |
|         | 600                    | 42 ± 4                 | -8.1 ± 0.4        |
|         | 5000                   | 41 ± 6                 | -5.2 ± 0.2        |
| Api1-17 | 100                    | 53 ± 6                 | 3.2 ± 0.4         |
|         | 600                    | 49 ± 6                 | -4.5 ± 0.1        |
|         | 5000                   | 46 ± 8                 | -0.64 ± 0.02      |
| Api1-16 | 100                    | 42 ± 2                 | 3.6 ± 0.3         |
|         | 600                    | 36 ± 4                 | -4.4 ± 0.2        |
|         | 5000                   | 35 ± 5                 | 1.9 ± 0.1         |
Table S8. Precision and accuracy of the optimized MRM method applied to murine heparin plasma samples calculated from three triplicates repeated on three consecutive days.

| Peptide | Concentration [ng/mL] | Precision Intraday | Precision Interday | Accuracy [%] |
|---------|------------------------|--------------------|-------------------|--------------|
|         |                        | 50                 | 250               | 1000         | 5000         |
| Api88   |                        |                    |                   |              |              |
|         |                        | 9 – 10%            | 1%                | 104 ± 6      |
|         | 250                    | 1 – 2%             | 1%                | 94 ± 1       |
|         | 1000                   | 1 – 2%             | 1%                | 99 ± 2       |
|         | 5000                   | 1 – 2%             | 1%                | 100 ± 1      |
| Api137  |                        | 50                 | 250               | 1000         | 5000         |
|         |                        | 8 – 11%            | 3%                | 116 ± 17     |
|         | 250                    | 4 – 7%             | 3%                | 89 ± 6       |
|         | 1000                   | 1 – 2%             | 2%                | 89 ± 1       |
|         | 5000                   | 1 – 2%             | 3%                | 100 ± 1      |
| Api1-17 |                        | 50                 | 250               | 1000         | 5000         |
|         |                        | 5 – 18%            | 1%                | 98 ± 13      |
|         | 250                    | 3 – 6%             | 1%                | 97 ± 5       |
|         | 1000                   | 1 – 2%             | 2%                | 100 ± 3      |
|         | 5000                   | 1 – 2%             | 3%                | 100 ± 1      |
| Api1-16 |                        | 50                 | 250               | 1000         | 5000         |
|         |                        | 12 – 14%           | 3%                | 94 ± 11      |
|         | 250                    | 2 – 4%             | 3%                | 94 ± 3       |
|         | 1000                   | 1 – 4%             | 1%                | 101 ± 1      |
|         | 5000                   | 1 – 5%             | 3%                | 101 ± 1      |
Table S9. LODs and LOQs determined for Api88, Api137, and their two main metabolites in urine and organ homogenates. The sample volumes of urine and liver, kidney, and brain homogenates were 10, 10, 25, and 50 µL, respectively.

| Peptide  | LODs / LOQs [ng/mL] | Plasma | Liver | Kidney | Brain | Urine |
|----------|----------------------|--------|-------|--------|-------|-------|
| Api88    | 55 / 59 (74 / 78)#   | 216 / 246 | 87 / 101 | 43 / 56 | 197 / 213 |
| Api137   | 35 / 39              | 191 / 266 | 76 / 93 | 18 / 34 | 69 / 114 |
| Api1-17  | 14 / 19              | 50 / 85  | 45 / 100 | 6 / 33  | 27 / 58  |
| Api1-16  | 16 / 26              | 77 / 232 | 33 / 42 | 13 / 18 | 35 / 107 |

# Studies using intravenous and intraperitoneal administrations were performed at different times and thus slightly different LODs/LOQs determined for each analysis period were applied.
Table S10. Peptide amounts recovered in mice samples after intraperitoneal administration of Api88. Quantities were corrected by the recovery rates determined for plasma and each organ homogenate (Table S12). Absolute quantities were calculated by assuming a total plasma volume of 25 mL/kg mouse weight and organ volumes determined in mL corresponded to the measured organ weights in g. Percentages of mean injected peptide amounts recovered in respective samples are provided in brackets.

| Dose | Time [min] | Mean mouse weight [g] | Mean injected peptide amount [µg] | Peptide amount [µg] |
|------|------------|-----------------------|-----------------------------------|--------------------|
|      |            |                       |                                   | Blood   | Liver   | Kidney  | Urine   | Total   |
| 5 mg/kg |          |                       |                                   | 0.9     | 1.1     | 0.9     | 0 – 0.009 | 2.9 (2.1%) |
| 20     | 10         | 27.8                  | 138.9                             | (0.7%)  | (0.8%)  | (0.6%)  | (0 – 0.006%) | 2.8 (2.0%) |
|        | 20         | 28.3                  | 141.6                             | 0.8     | 1.2     | 0.8     | 0        | 1.7 (1.3%) |
|        | 30         | 27.3                  | 136.5                             | 0.5     | 0.6     | 0.7     | 0        | 0.3 (0.2%) |
|        | 60         | 26.3                  | 131.6                             | 0.1     | 0       | 1.1     | 0.006    | 0.2 (0.1%) |
|        | 90         | 27.4                  | 137.0                             | 0       | 0       | 0.2     | 0        | 0.2 (0.1%) |
| 20 mg/kg |          |                       |                                   | 6.2     | 1.7     | 1.8     | 0 – 0.02 | 9.7 (1.5%) |
|        | 10         | 32.3                  | 646.9                             | (1.0%)  | (0.3%)  | (0.3%)  | (0 – 0.003%) | 6.4 (1.0%) |
|        | 20         | 30.6                  | 612.9                             | 3.3     | 1.2     | 1.9     | 0.004 – 0.02 | 5.3 (0.9%) |
|        | 30         | 29.4                  | 588.9                             | 3.2     | 1.2     | 0.8     | 0.008 – 0.013 | 1.0 (0.1%) |
|        | 60         | 31.8                  | 636.6                             | 0.4     | 0       | 0.6     | 0 – 0.03 | 1.0 (0.2%) |
|        | 90         | 30.3                  | 606.9                             | 0.1     | 0       | 0.2     | 0 – 0.05 | 0.3 (<0.1%) |
Table S11. Peptide amounts recovered in mice samples after intraperitoneal administration of Api137. Quantities were corrected by the recovery rates determined for plasma and each organ homogenate (Table S12). Absolute quantities were calculated by assuming a total plasma volume of 25 mL/kg mouse weight and organ volumes determined in mL corresponded to the measured organ weights in g. Percentages of mean injected peptide amounts recovered in respective samples are provided in brackets.

| Dose     | Time [min] | Mean mouse weight [g] | Mean injected peptide amount [µg] | Peptide amount [µg] | Blood   | Liver | Kidney | Urine      | Total     |
|----------|------------|-----------------------|-----------------------------------|---------------------|---------|-------|--------|------------|-----------|
|          |            |                       |                                   |                     |         |       |        |            |           |
| 5 mg/kg  | 10         | 31.5                  | 157.4                             | 0.2 (0.1%)          | 0.3 (0.2%) | 2.4 (1.5%) | 0 – 0.005 | 3.0 (1.9%)  |
|          | 20         | 31.2                  | 155.9                             | 0.1 (0.1%)          | 0.1 (0.1%) | 2.5 (1.6%) | 0 – 0.01   | 2.8 (1.8%)  |
|          | 30         | 27.2                  | 136.1                             | 0.1 (0.1%)          | 0.1 (0.1%) | 1.4 (1.0%) | 0.004 – 0.04 | 1.6 (1.2%)  |
|          | 60         | 27.5                  | 137.6                             | <0.1 (<0.1%)        | 0 (0%)    | 0      | 0       | <0.1 (<0.1%) | <0.1 (<0.1%) |
|          | 90         | 28.1                  | 140.6                             | <0.1 (<0.1%)        | 0 (0%)    | 0      | 0 – 0.02 | <0.1 (<0.1%) | <0.1 (<0.1%) |
| 20 mg/kg | 10         | 30.1                  | 601.4                             | 3.5 (0.6%)          | 0.8 (0.1%) | 8.6 (1.4%) | 0         | 12.8 (2.1%) |
|          | 20         | 30.4                  | 607.4                             | 1.9 (0.3%)          | 0.5 (0.1%) | 10.1 (1.7%) | 0 – 0.004  | 12.5 (2.1%) |
|          | 30         | 32.1                  | 641.7                             | 0.5 (0.1%)          | 0.2 (<0.1%) | 7.9 (1.2%) | 0 – 0.004  | 8.5 (1.3%)  |
|          | 60         | 29.6                  | 592.3                             | 0.2 (<0.1%)         | 0.2 (<0.1%) | 5.2 (0.9%) | 0 – 0.006  | 5.5 (0.9%)  |
|          | 90         | 28.5                  | 569.1                             | <0.1 (<0.1%)        | 0 (0%)    | 0.1 (<0.1%) | 0 – 0.006  | 0.1 (<0.1%)  |
Table S12. Recovery rates of Api88 and Api137 determined in plasma and organ homogenates (n = 3) collected from untreated NMRI mice. Solutions of Api88 (blood: 10 µL, 25 and 100 µg/mL, brain: 500 µL, 0.2 or 1 µg/mL, liver: 500 µL, 2 or 5 µg/mL, kidneys: 500 µL, 20 or 60 µg/mL) and Api137 (blood: 10 µL, 2.5 and 10 µg/mL, brain: 500 µL, 0.2 or 2 µg/mL, liver: 500 µL, 1 or 2.5 µg/mL, kidneys: 500 µL, 20 or 60 µg/mL) were added prior to homogenization and centrifugation. Peptide concentrations were calculated based on the assumption that peptides were equally distributed in plasma (one third of the 2.5 mL blood volume of mice) and present only in the supernatant of the homogenates, i.e. neglecting peptide contents remaining in the pellets. The total peptide amount in mice was calculated from the recovery rates shown in bold.

| Sample   | Api88          |         | Api137          |         |
|----------|---------------|---------|-----------------|---------|
|          | High concentration | Low concentration | High concentration | Low concentration |
| Plasma   | (48 ± 6)%     | (49 ± 5)%   | (77 ± 1)%       | (78 ± 4)%   |
| Kidney   | (14.0 ± 0.2)% | (16.7 ± 6)% | (2.7 ± 0.7)%    | (3.5 ± 0.4)% |
| Liver    | (34 ± 3)%     | (45 ± 5)%   | (47 ± 4)%       | (49 ± 4)%   |
| Brain    | 0%            | (89 ± 24)%  | 0%              | (42 ± 4)%   |
Table S13. Metabolites of Api88 and Api137 determined in homogenates prepared from kidneys collected 10 min, 30 min, and 60 min after intraperitoneal peptide administration. Kidney samples obtained from three mice per time point were pooled and analyzed by nanoRP-UPLC-ESI-LTQ-Orbitrap XLTM-MS. Api88 and Api137 were not detected in any sample.

| Metabolite | Charge state | m/z       | Retention time [min] | sequence<sup>c</sup>                  |
|------------|--------------|-----------|----------------------|---------------------------------------|
| 1-9        | 2            | 592.85    | 19.1                 | guan-ONNRPVYIP-OH                     |
| 1-11       | 3            | 479.95    | 12.0                 | guan-ONNRPVYIPRP-OH                  |
| 1-13       | 4            | 423.51    | 13.2                 | guan-ONNRPVYIPRPRP-OH                |
| 1-14       | 4            | 447.77    | 13.9                 | guan-ONNRPVYIPRPRPP-OH               |
| 1-16       | 4/5          | 506.30/405.24 | 14.0   | guan-ONNRPVYIPRPRP-OH               |
| 2-16       | 4            | 453.26    | 13.9                 | H-NNRPVYIPRPRP-OH                    |
| 7-16       | 3            | 410.57    | 12.6                 | H-YIPRPRP-OH                         |
| 8-14       | 2            | 416.76    | 12.0                 | H-IPRPRP-OH                          |
| 8-18       | 3            | 445.60<sup>a</sup>/445.94<sup>b</sup> | 13.6<sup>a</sup>/13.5<sup>b</sup> | H-IPRPRPPRL-NH<sub>2</sub>/OH           |
| 9-18       | 3            | 407.92<sup>a</sup> | 16.7<sup>a</sup> | H-PRPRPPRL-NH<sub>2</sub>/OH           |
| 12-18      | 2            | 436.27<sup>a</sup>/436.76<sup>b</sup> | 11.1<sup>a</sup>/10.8<sup>b</sup> | H-RPPRL-NH<sub>2</sub>/OH              |

<sup>a</sup> Retention time and m/z obtained for a C-terminal metabolite of Api88
<sup>b</sup> Retention time and m/z obtained for a C-terminal metabolite of Api137
<sup>c</sup> C-terminal sequences were always amidated and free acids when derived from Api88 and Api137, respectively.

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Figure S1. ESI-QqLIT-MS (A and C) and -MS/MS (B and D; [M+3H]+3+) of Api88 (A and B) and Api137 (C and D). Api88 and 137 were infused with a syringe pump (5 µL/min). Fragmentation relied on collision potential setting of 45 V (Api88) and 40 V (Api137) using nitrogen as collision gas. Mass shifts indicating neutral losses of ammonia and water are indicated by asterisks and apostrophes, respectively.
Figure S2. Influence of pH on signal intensities in MRM. Mean signal intensities (n = 3) of different precursor charge states were determined for Api88 (A) and Api137 (B) for a pH range from 2.6 to 4.0 in the presence of formate/formic acid. Peptides were dissolved in 25% (v/v) aq. acetonitrile containing 26 mmol/L formate buffer, infused (5 µL/min), and analyzed in Q1 scan mode on a 4000 QTRAP®.
Figure S3. Chromatograms of Api88 (black) and Api137 (red) (A and B) and their isotope-labeled internal standards (C and D, respectively) recorded by the optimized MRM. Plasma extracts (10 µL) were analyzed without (A and C) and after addition of native (0.1 µg/mL, B) or isotope-labelled Api88 and Api137 (2.5 µg/mL, D).
Figure S4. Pharmacokinetics of Api88 (A), Api137 (B), and metabolites 1-17 and 1-16 (C and D) after intraperitoneal administration at doses of 5 mg/kg BW. Peptides were quantified by RPC-ESI-QqLIT-MS/MS in plasma samples collected at 10, 20, 30, 60, and 90 min post injection (n = 7).
Figure S5: Pharmacokinetics of Api88 (A and C) and Api137 (B and D) intraperitoneally administered at doses of 20 mg/kg BW (A and B) and 5 mg/kg BW (C and D). Graphs and formula of exponential fittings used to determine the pharmacokinetic parameters were calculated with PKSolver. The exponential elimination time range was automatically set by the software. Peptides were quantified by RPC-ESI-QqLIT-MS/MS in plasma samples collected at 10, 20, 30, 60, and 90 min post injection (n = 7).
Figure S6. Peak areas of Api88 (A), Api137 (B), and the respective metabolites were determined in heparin plasma samples using the extracted ion chromatograms (XICs, $m/z \pm 0.01$). Blood samples were collected 10, 30, and 60 min after intraperitoneal administration of Api88 or Api137 (three mice per time point), pooled, and analyzed by nanoRP-UPLC-LTQ-Orbitrap XL™-MS. Peak areas represent the sum of all peak areas of a charge series of a given peptide or metabolite normalized to the total peak areas. Shown are metabolites with peak areas larger than $10^6$ counts.