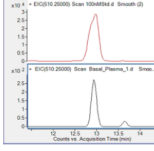


Supplementary Information

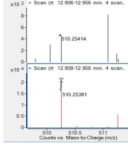
**A class of secreted mammalian peptides with potential to expand cell-cell
communication**

Wiggenhorn et al.

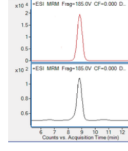
a. CAP-LAMB2
EIC of MS1



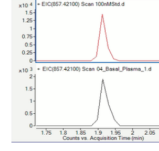
MS1 spectra



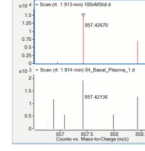
MS2 - 510.3>249.1 (b2+)



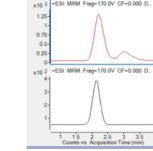
b. CAP-ADAMTS18
EIC of MS1



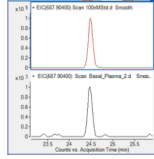
MS1 spectra



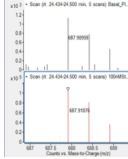
MS2 - 215.1>136.9 (b5++++)



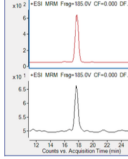
c. CAP-VIP
EIC of MS1



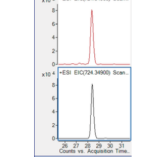
MS1 spectra



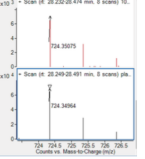
MS2 - 687.9>608.9 (a11++)



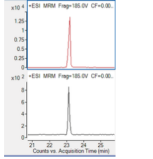
d. CAP-TAC1
EIC of MS1



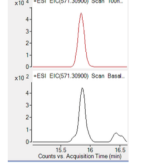
MS1 spectra



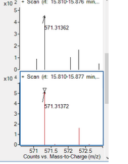
MS2 - 724.3>463.2 (b4+)



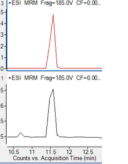
e. CAP-OBSCN
EIC of MS1



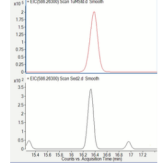
MS1 spectra



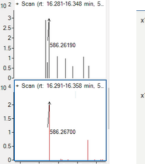
MS2 - 571.3>248.1 (y2+)



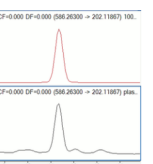
f. CAP-FGF5
EIC of MS1



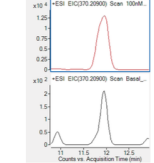
MS1 spectra



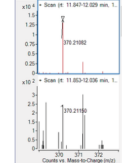
MS2 - 586.3>202.1 (y2+)



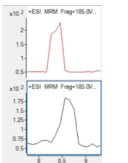
g. CAP-EMILIN1
EIC of MS1



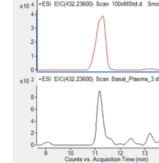
MS1 spectra



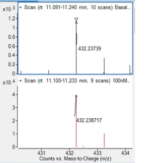
MS2 - 370.2>183.1 (b2+)



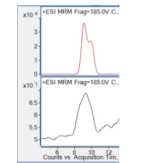
h. CAP-WNT3A
EIC of MS1



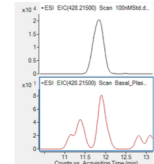
MS1 spectra



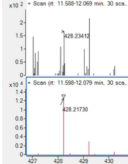
MS2 - 432.3>157.1 (z1+)



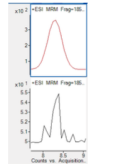
i. CAP-THBS4
EIC of MS1



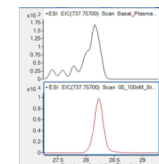
MS1 spectra



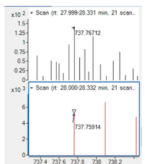
MS2 - 428.2>225.1 (b2+)



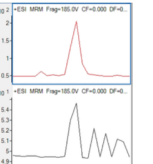
j. CAP-CSF1
EIC of MS1



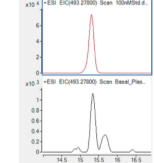
MS1 spectra



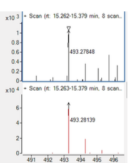
MS2 - 737.8>976.6 (b18++)



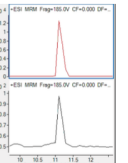
k. CAP-COL27A1
EIC of MS1



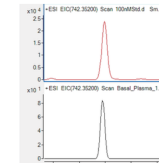
MS1 spectra



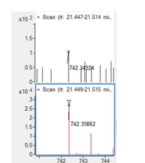
MS2 - 493.3>212.1 (y2+)



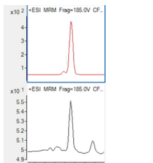
l. CAP-DEFA-RS7
EIC of MS1



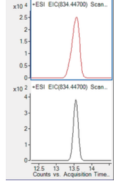
MS1 spectra



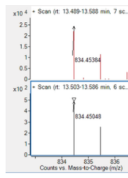
MS2 - 742.4>539.2 (b6+)



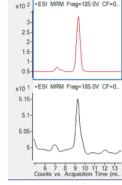
m. CAP-METTL24
EIC of MS1



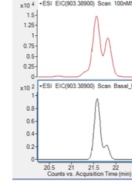
MS1 spectra



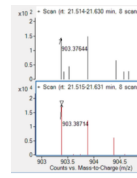
MS2 - 834.4>475.2 (b4+)



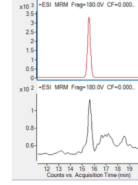
n. CAP-TENM1
EIC of MS1



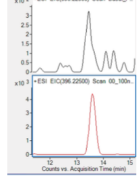
MS1 spectra



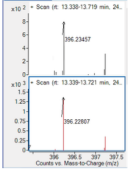
MS2 - 903.4>886.3 (z7+)



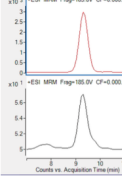
o. CAP-CA15
EIC of MS1



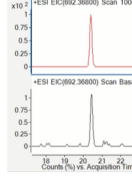
MS1 spectra



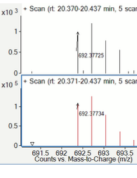
MS2 - 396.2>188.1 (v2+)



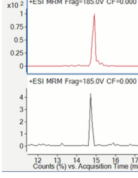
p. CAP-ANGPTL4
EIC of MS1



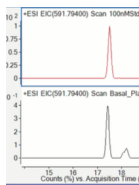
MS1 spectra



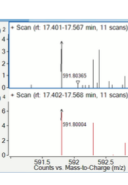
MS2 - 692.4>453.2 (b4+)



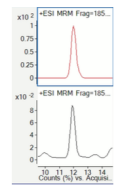
q. GnRH
EIC of MS1



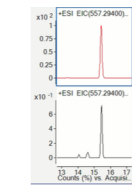
MS1 spectra



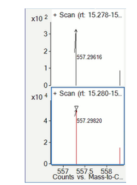
MS2 - 591.8>249.1 (b2+)



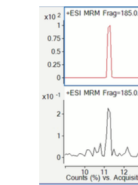
r. CAP-CNPY4
EIC of MS1



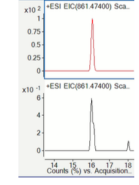
MS1 spectra



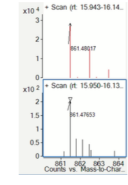
MS2 - 557.3>439.2 (b4+)



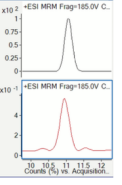
s. CAP-NTN4
EIC of MS1



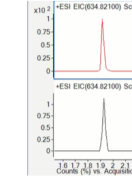
MS1 spectra



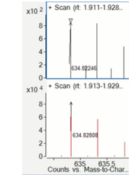
MS2 - 861.5>249.1 (b2+)



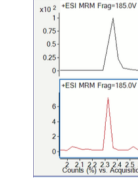
t. CAP-GDNF
EIC of MS1



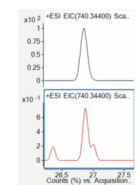
MS1 spectra



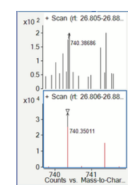
MS2 - 634.8>325.2 (b4+)



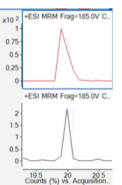
u. CAP-TAC4
EIC of MS1



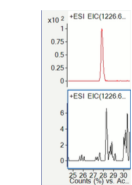
MS1 spectra



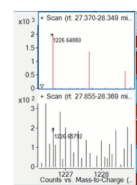
MS2 - 740.3>479.2 (b4+)



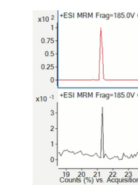
v. CAP-DEFB1
EIC of MS1



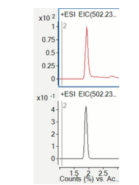
MS1 spectra



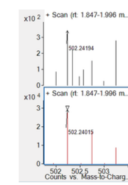
MS2 - 1226.6>795.4 (b8+)



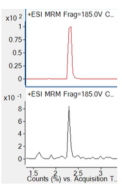
w. CAP-PRG4
EIC of MS1



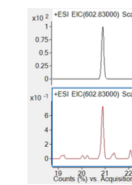
MS1 spectra



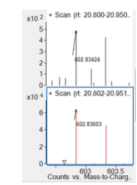
MS2 - 502.2>382.2 (y6++)



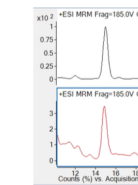
x. CAP-PLAG2G2A
EIC of MS1



MS1 spectra

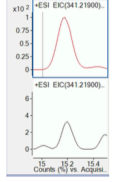


MS2 - 602.8>231.1 (a2+)



y. CAP-FGF18

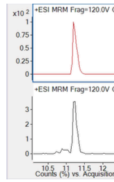
EIC of MS1



MS1 spectra

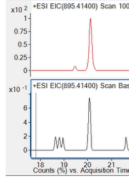


MS2 - 341.2>131.1 (y1+)



z. CAP-LY86

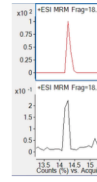
EIC of MS1



MS1 spectra

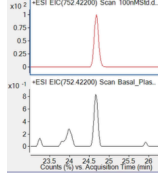


MS2 - 903.4>484.3 (b4+)

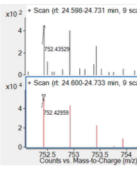


aa. CAP-VIP-2

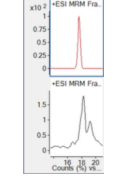
EIC of MS1



MS1 spectra

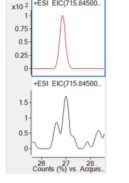


MS2 - 752.4>132.1 (y1+)

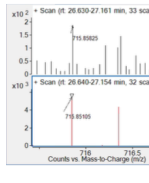


bb. CAP-PRSS36

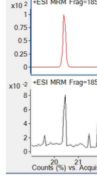
EIC of MS1



MS1 spectra

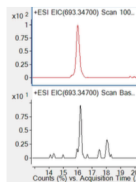


MS2 - 715.8>244.1 (y2+)



cc. CAP-GNAS

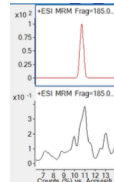
EIC of MS1



MS1 spectra

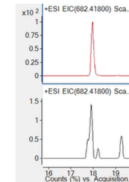


MS2 - 693.3>325.2 (y3+)

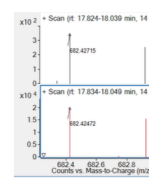


dd. CAP-GDF15

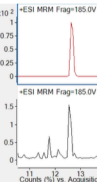
EIC of MS1



MS1 spectra

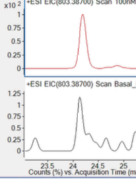


MS2 - 682.4>354.2 (b3+)

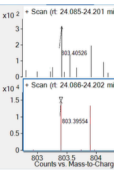


ee. CAP-TAC3

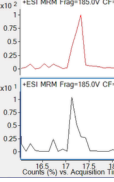
EIC of MS1



MS1 spectra

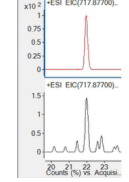


MS2 - 803.4>729.4 (b12++)

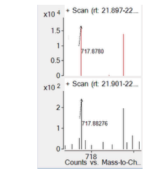


ff. CAP-EGFL8

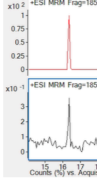
EIC of MS1



MS1 spectra

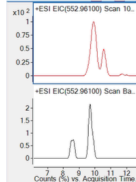


MS2 - 717.9>859.4 (b7+)

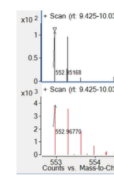


gg. CAP-COL5A2-2

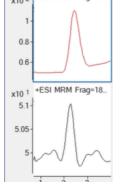
EIC of MS1



MS1 spectra

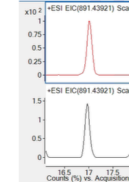


MS2 - 553.0>454.2 (b4+)

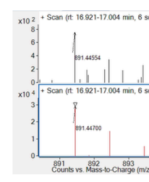


hh. CAP-MST1

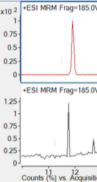
EIC of MS1



MS1 spectra

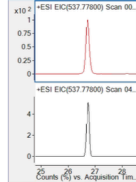


MS2 - 891.4 >306.2 (y2+)



ii. CAP-ALKAL2

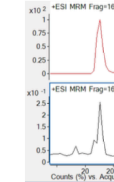
EIC of MS1



MS1 spectra

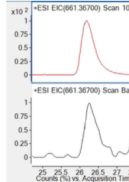


MS2 - 1074.5>944.4 (b9+)

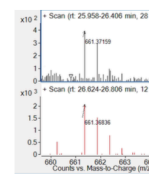


jj. CAP-GDF6

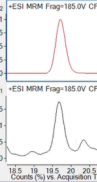
EIC of MS1



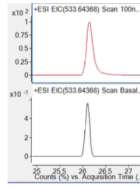
MS1 spectra



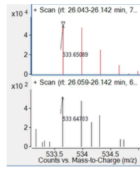
MS2 - 661.4>209.1 (b2+)



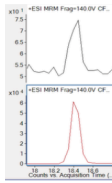
kk. CAP-GCG
EIC of MS1



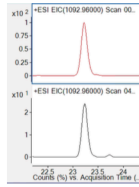
MS1 spectra



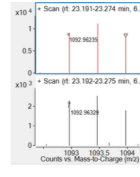
MS2 - 533.6>129.1 (c1+)



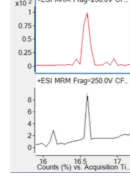
ll. Gastrin
EIC of MS1



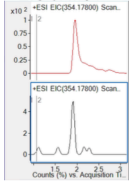
MS1 spectra



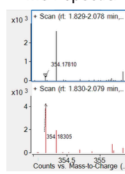
MS2 - 1093.0>1010.9 (b16++)



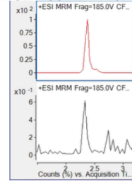
mm. CAP-COL5A2
EIC of MS1



MS1 spectra

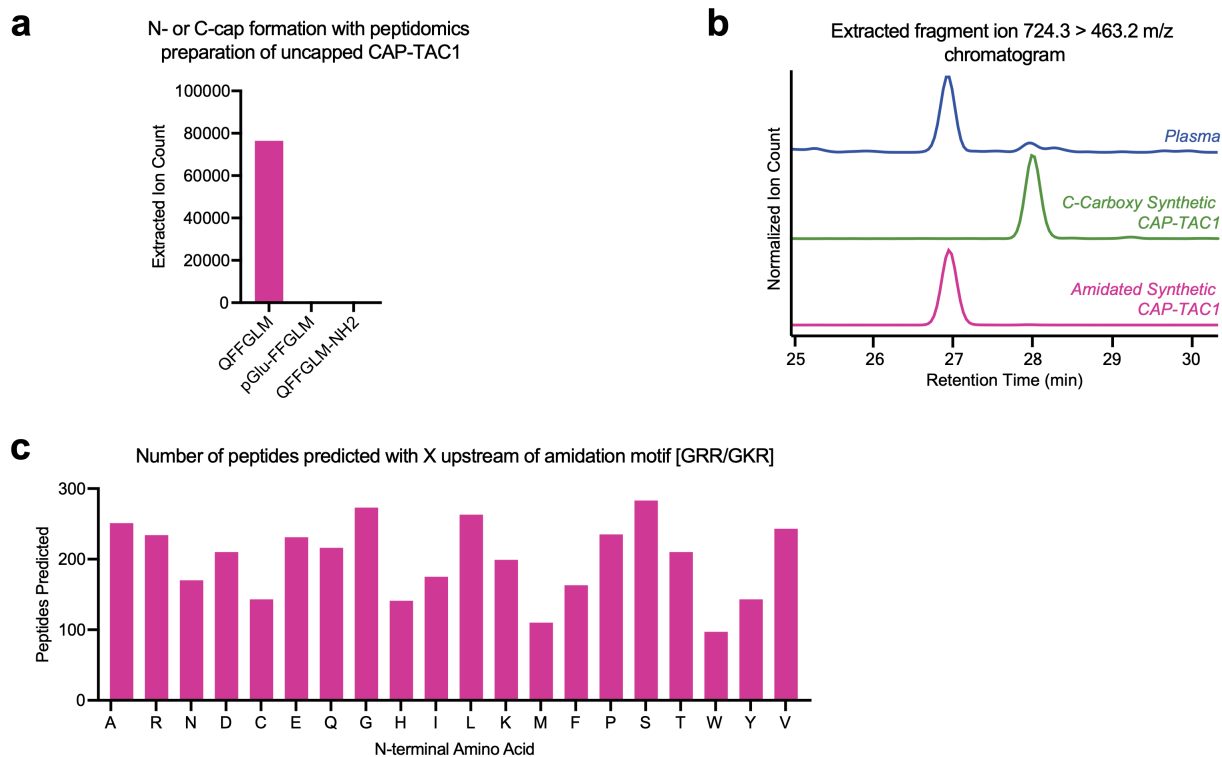


MS2 - 354.2>115.1 (y1+)



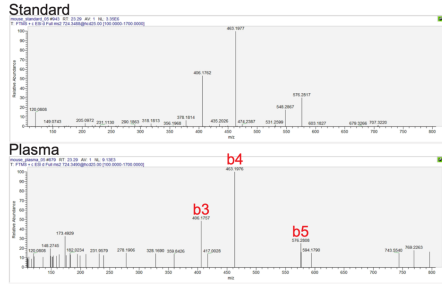
— Mouse Plasma — Synthetic Standard

Supplementary Figure 1. All raw MS1 chromatograms, MS1 spectra, and MRM transition chromatograms for detected mouse capped peptides. a-mm The extracted parent ion chromatogram, the MS1 spectra, and MRM fragment ion chromatogram both in mouse plasma (black) and of the synthetic standards (red).

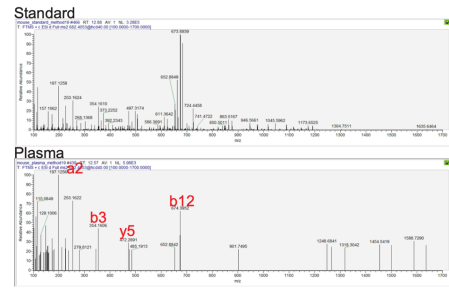


Supplementary Figure 2. Additional capped peptide prediction analysis and validation of capped peptide extraction and detection. **a** Extracted ion count of QFFGLM (uncapped CAP-TAC1, $m/z = 742.359$), pGlu-FFGLM ($m/z = 725.33$), or QFFGLM-NH₂ ($m/z = 741.375$) after peptidomics preparation of 10 nM QFFGLM (uncapped) synthetic standard. **b** Chromatograms of CAP-TAC1 b₄₊ ion (724.3>463.2) in mouse plasma, 100 nM C-carboxy synthetic CAP-TAC1 standard, and 100 nM C-amidated synthetic CAP-TAC1. **c** Number of peptides predicted when the capped peptide prediction algorithm modified for X N-terminal amino acid upstream of the amidation motif. Source data are provided as a Source Data file.

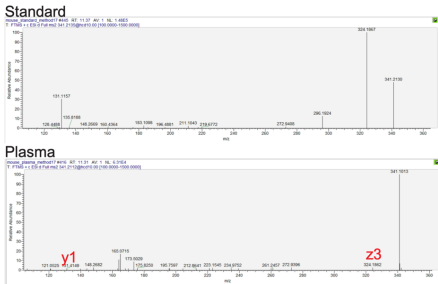
a. CAP-TAC1



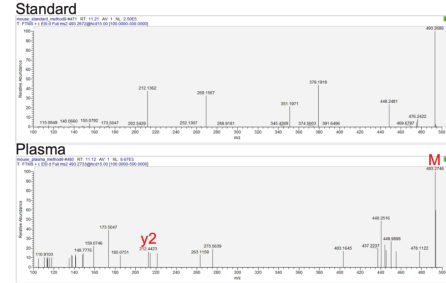
b. CAP-GDF15



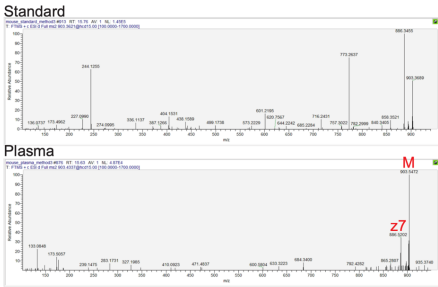
c. CAP-FGF18



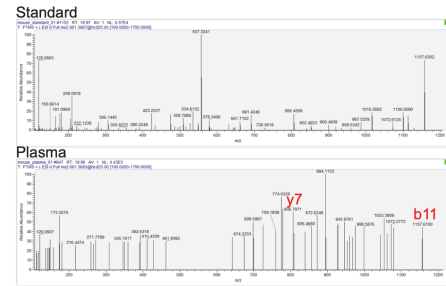
d. CAP-COL27A1



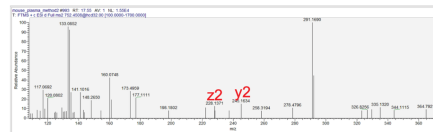
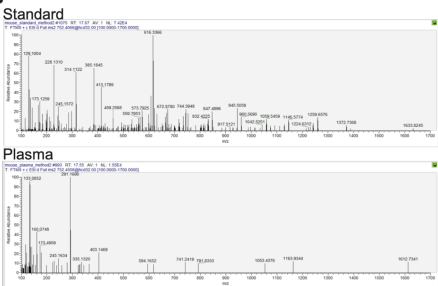
e. CAP-TENM1



f. CAP-GDF6

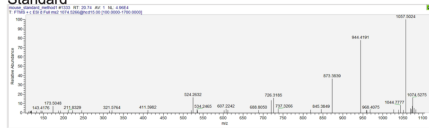


g. CAP-VIP-2

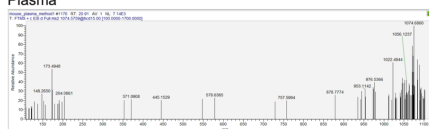


h. CAP-ALKAL2

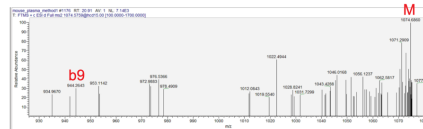
Standard



Plasma

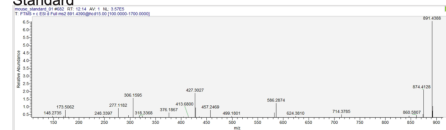


Zoomed >

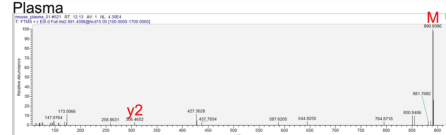


i. CAP-MST1

Standard

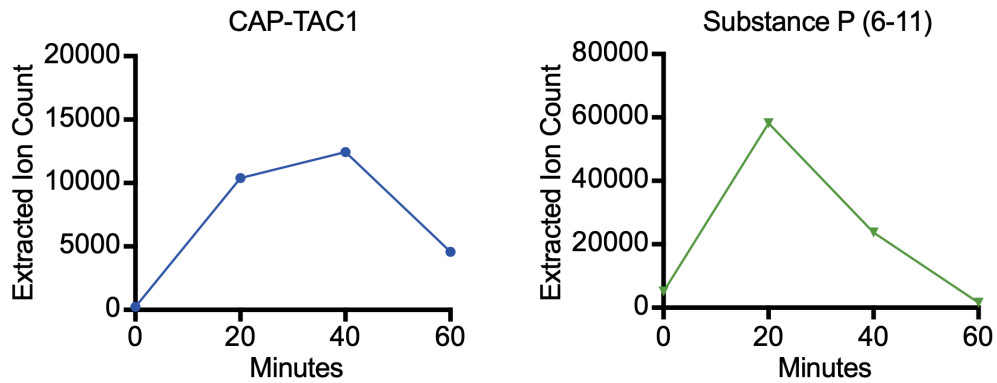


Plasma

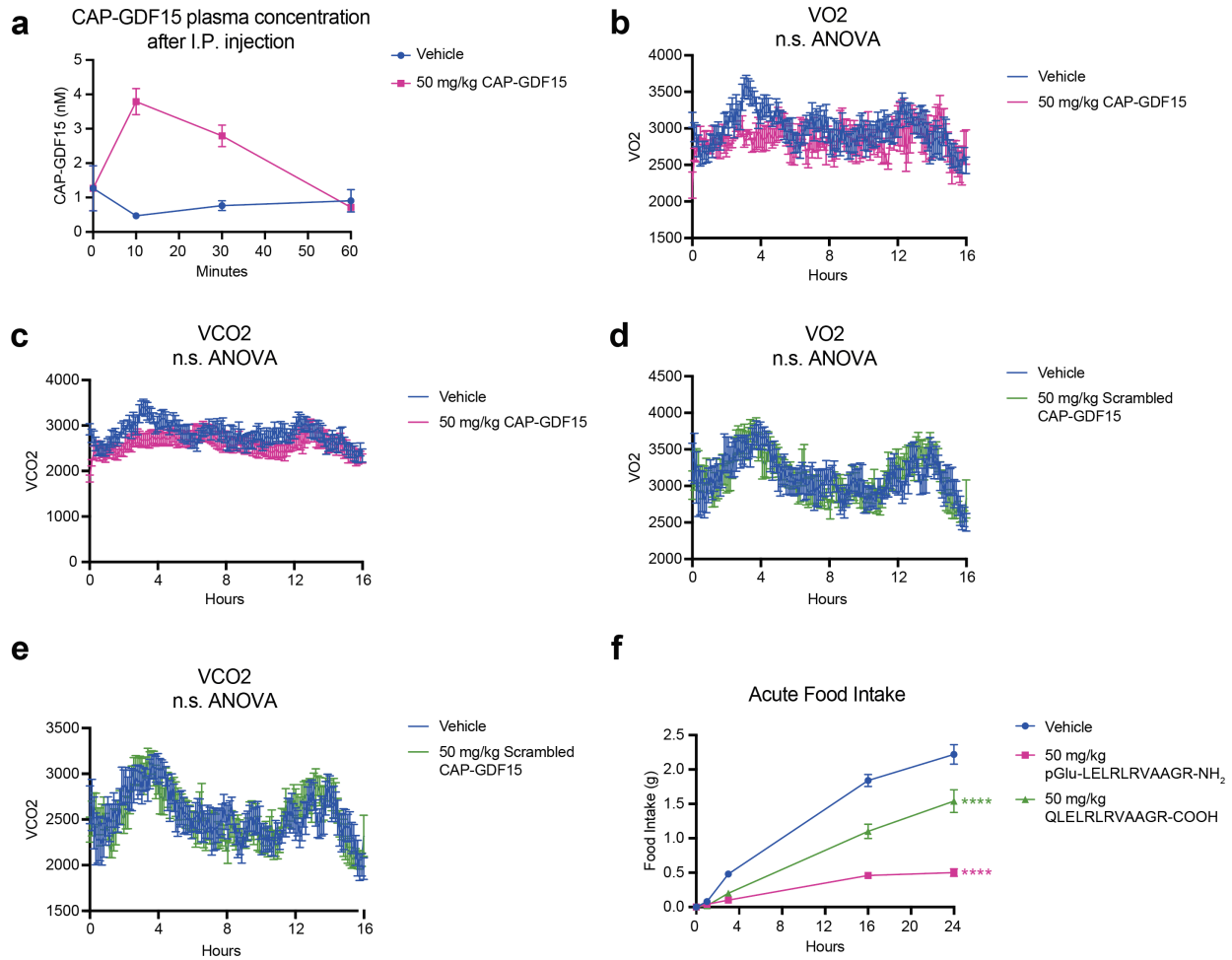


Supplementary Figure 3. Additional MS2 spectra for mouse capped peptides. a-i MS2 spectra of 9 mouse capped peptides both in mouse plasma and of the synthetic standards.

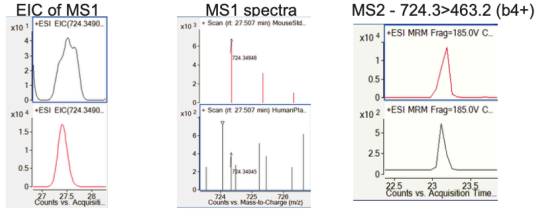
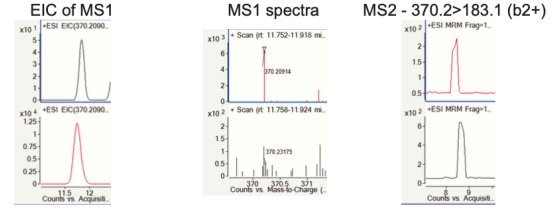
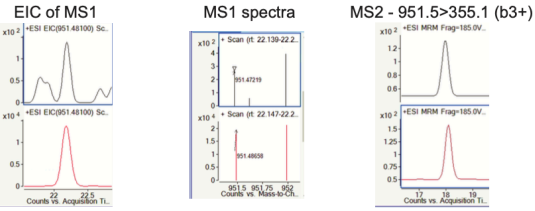
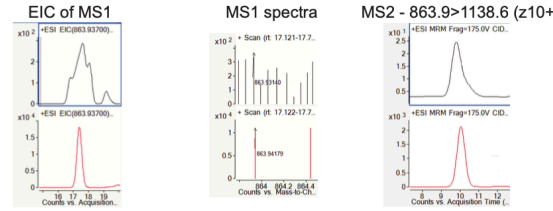
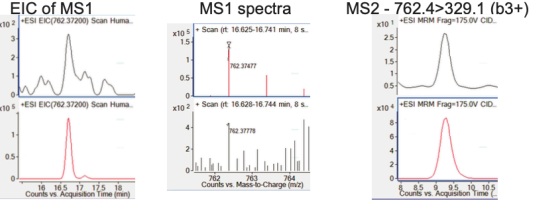
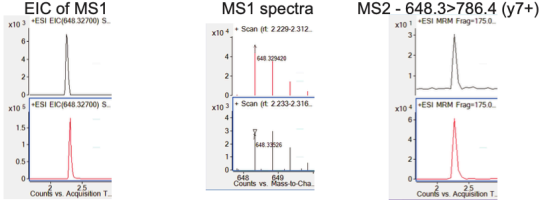
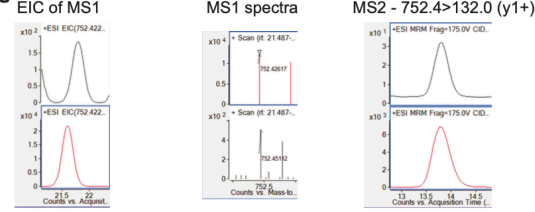
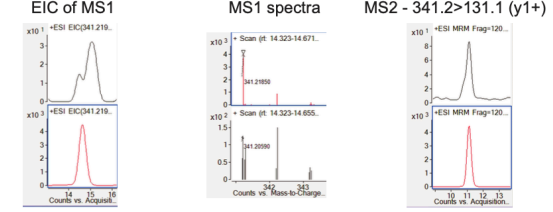
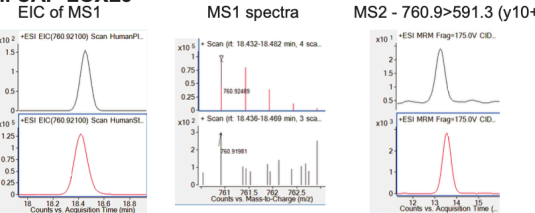
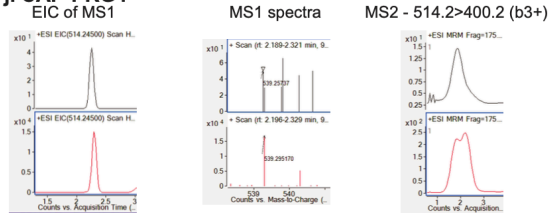
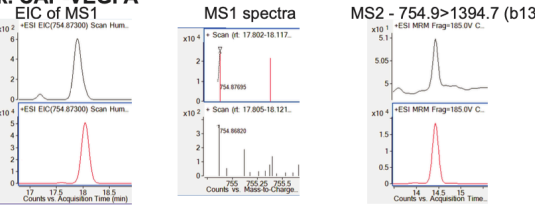
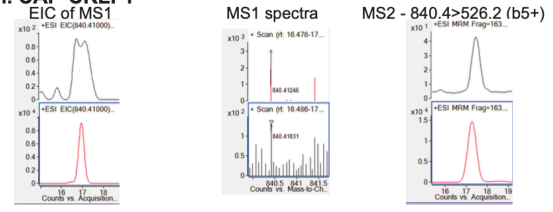
CAP-TAC1 and Substance P (6-11) production from
from substance in plasma



Supplementary Figure 4. CAP-TAC1 and substance P (6-11) production during substance P degradation. Relative levels of CAP-TAC1 and substance P (6-11) in mouse plasma following incubation at 37°C for the indicated time. N=2 biological replicates per concentration per time point. Data are shown as means. Source data are provided as a Source Data file.

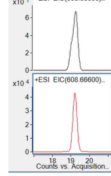


Supplementary Figure 5. Additional measurements from metabolic chambers of mice treated with CAP-GDF15. **a** CAP-GDF15 plasma concentration after administration of CAP-GDF15 (50 mg/kg, intraperitoneal) or vehicle control. **b-c** VO₂ (**b**) and VCO₂ (**c**) of 12-16-week diet-induced obese male mice following a single treatment of CAP-GDF15 (50 mg/kg, intraperitoneal) or vehicle control. **d-e** VO₂ (**d**) and VCO₂ (**e**) of 12-16-week diet-induced obese male mice following a single treatment of scrambled CAP-GDF15 (50 mg/kg, intraperitoneal, scrambled sequence: pQGLEALRRLRV-NH₂) or vehicle control. **f** Acute food intake of 12-16-week diet-induced obese male mice following a single treatment of CAP-GDF15 (50 mg/kg, intraperitoneal), uncapped CAP-GDF15 (50 mg/kg, intraperitoneal), or vehicle control. For (**a**), N=3/group; (**b-c**), N=12/group; for (**d-e**), N=8/group; for (**f**), N=5/group. For (**a-f**), injection occurred at time T=0 (5:00pm). Data are shown as means ± SEM. For (**b-i**), p values were calculated by two-way ANOVA. Source data are provided as a Source Data file.

a. CAP-TAC1**b. CAP-EMILIN1****c. CAP-F5****d. CAP-EDN3****e. CAP-Q59F13****f. CAP-GDNF****g. CAP-VIP****h. CAP-FGF18****i. CAP-LOXL3****j. CAP-PRG4****k. CAP-VEGFA****l. CAP-CRLF1**

m. CAP-FURIN

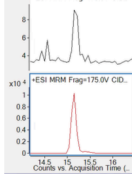
EIC of MS1



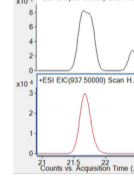
MS1 spectra



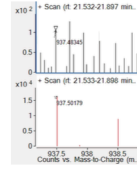
MS2 - 912.5>1363.7 (b11+)

**n. CAP-TG**

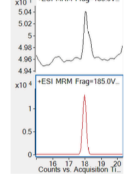
EIC of MS1



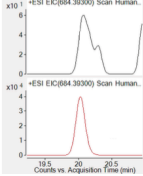
MS1 spectra



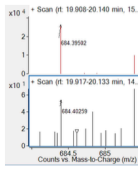
MS2 - 937.5>678.3 (b7+)

**o. CAP-MEGF6-2**

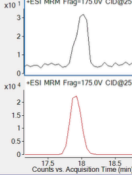
EIC of MS1



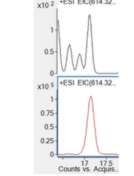
MS1 spectra



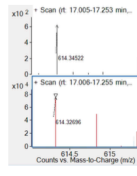
MS2 - 684.4>455.2 (b4+)

**p. CAP-IFNG-1**

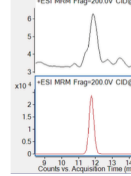
EIC of MS1



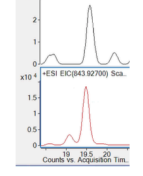
MS1 spectra



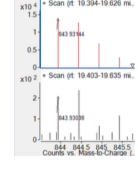
MS2 - 1227.6>486.3 (y5+)

**q. CAP-SIGLEC1**

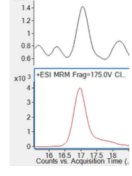
EIC of MS1



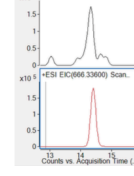
MS1 spectra



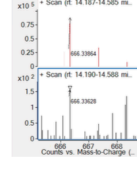
MS2 - 843.9>799.9 (b16++)

**r. CAP-CPAMD8-2**

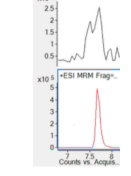
EIC of MS1



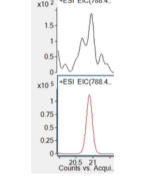
MS1 spectra



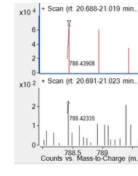
MS2 - 666.3>524.3 (a5+)

**s. CAP-CBLN2**

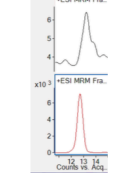
EIC of MS1



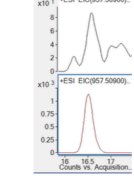
MS1 spectra



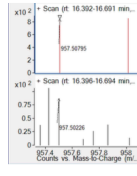
MS2 - 788.4>717.4 (a14++)

**t. CAP-CPAMD8-1**

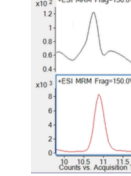
EIC of MS1



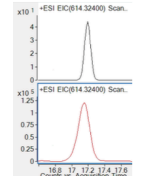
MS1 spectra



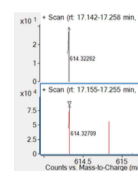
MS2 - 638.7>84.0 (a1+)

**u. CAP-P2MCH**

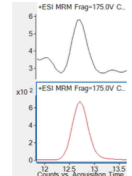
EIC of MS1



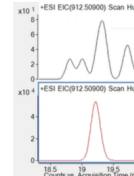
MS1 spectra



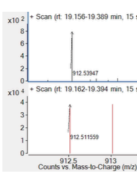
MS2 - 986.5>939.5 (b8+)

**v. CAP-LEFTY2**

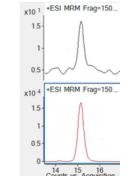
EIC of MS1



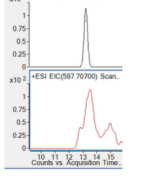
MS1 spectra



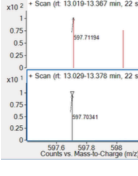
MS2 - 912.5>903.6 (b15+)

**w. CAP-OSM**

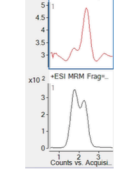
EIC of MS1



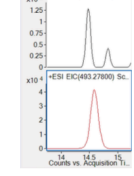
MS1 spectra



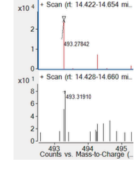
MS2 - 597.7>146.1 (y1+)

**x. CAP-COL27A1**

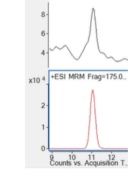
EIC of MS1



MS1 spectra

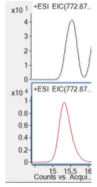


MS2 - 493.3>212.1 (y2+)

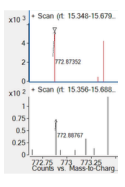


y. CAP-COL11A2

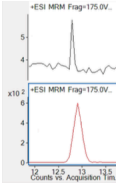
EIC of MS1



MS1 spectra

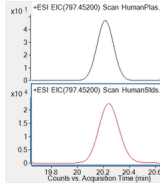


MS2 - 772.9>1192.6 (y13+)



z. CAP-MUC4

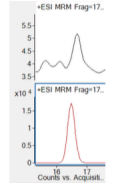
EIC of MS1



MS1 spectra

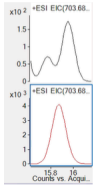


MS2 - 797.4>667.3 (b7+)

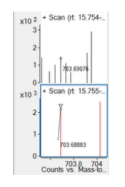


aa. CAP-F8

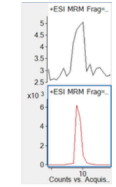
EIC of MS1



MS1 spectra

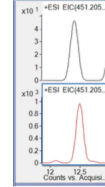


MS2 - 703.7>1110.6 (y10+)

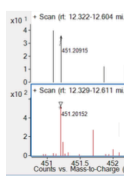


bb. CAP-BMP4

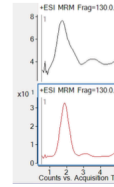
EIC of MS1



MS1 spectra

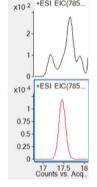


MS2 - 451.2>306.1 (b3+)



cc. CAP-COL4A4

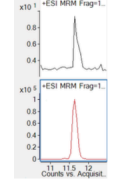
EIC of MS1



MS1 spectra

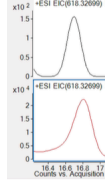


MS2 - 785.4>1160.6 (a12+)

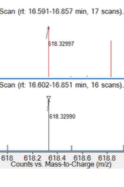


dd. CAP-LGALS9

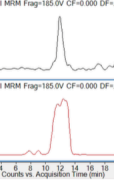
EIC of MS1



MS1 spectra

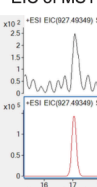


MS2 - 618.3>209.1 (b2+)

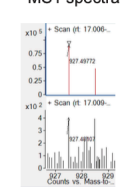


ee. CAP-LIPC

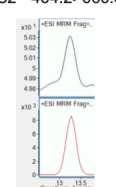
EIC of MS1



MS1 spectra

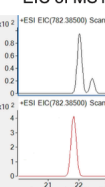


MS2 - 464.2>666.3 (b6+)

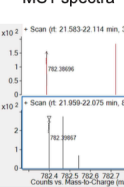


ff. CAP-WNT9A

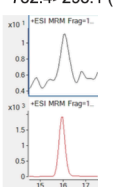
EIC of MS1



MS1 spectra

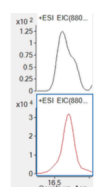


MS2 - 782.4>298.1 (b2+)

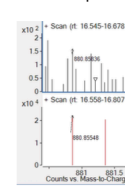


gg. CAP-VWF

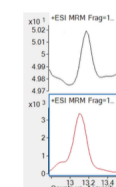
EIC of MS1



MS1 spectra

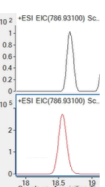


MS2 - 880.9>587.2 (y10++)

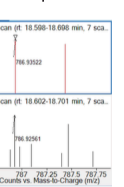


hh. CAP-THSD1

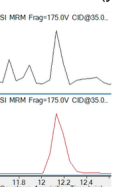
EIC of MS1



MS1 spectra

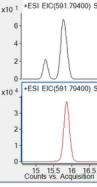


MS2 - 786.9>1006.5 (y8+)

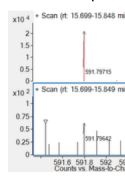


ii. GnRH

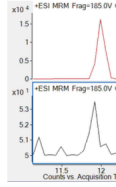
EIC of MS1



MS1 spectra

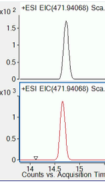


MS2 - 591.8>249.1 (b2+)



jj. CAP-GDF15-2

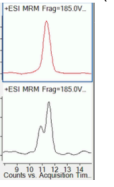
EIC of MS1

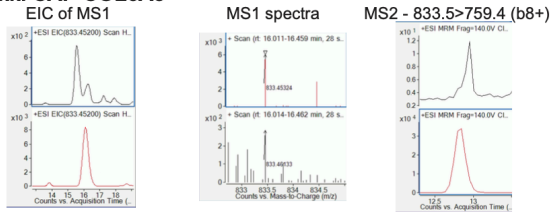
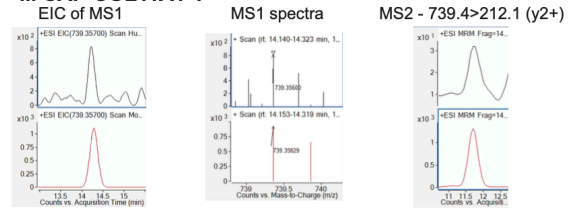
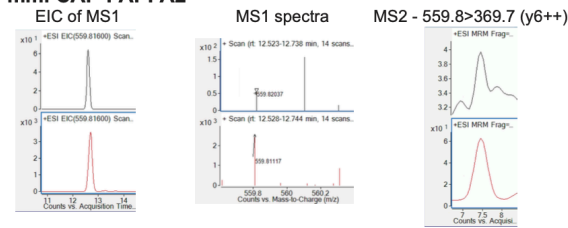
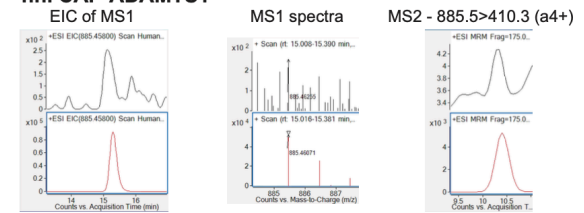
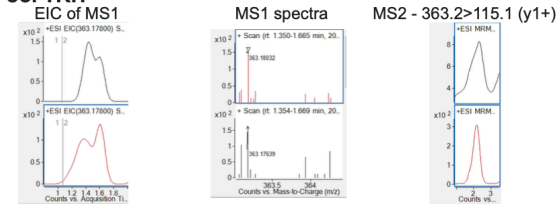
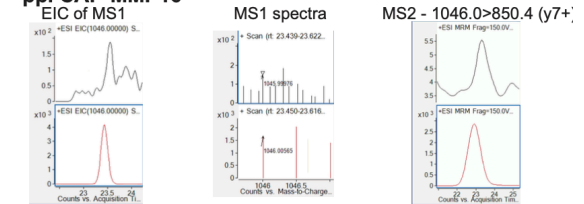
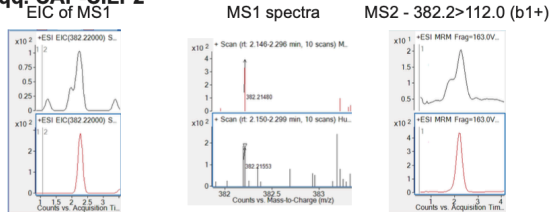
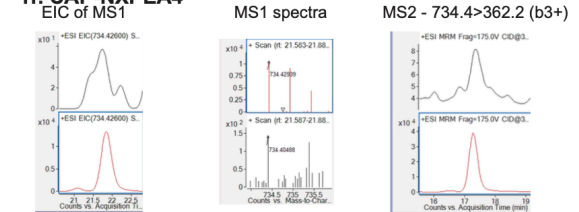
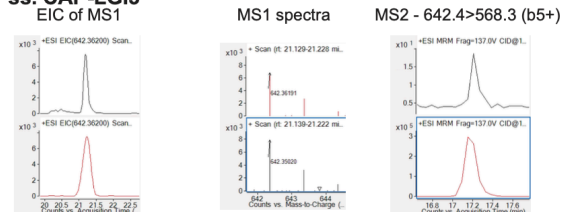


MS1 spectra



MS2 - 471.9>354.2 (b3+)

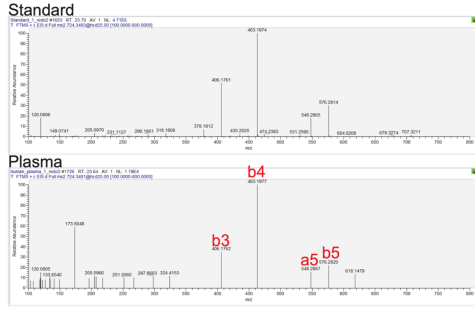


kk. CAP-COL6A3**ll. CAP-COL11A1-1****mm. CAP-PAPPA2****nn. CAP-ADAMTS1****oo. TRH****pp. CAP-MMP16****qq. CAP-CILP2****rr. CAP-NXPEA4****ss. CAP-LGI3**

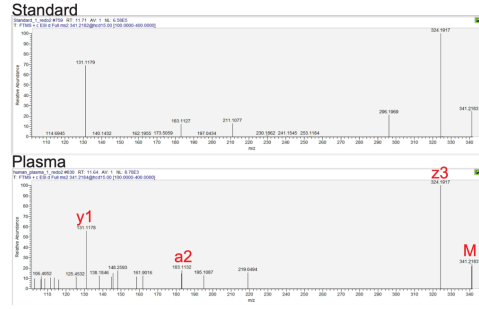
— Human Plasma — Synthetic Standard

Supplementary Figure 6. All raw MS1 chromatograms, MS1 spectra, and MRM transition chromatograms for detected human capped peptides. a-ss The extracted parent ion chromatogram, the MS1 spectra, and MRM fragment ion chromatogram both in human plasma (black) and of the synthetic standards (red).

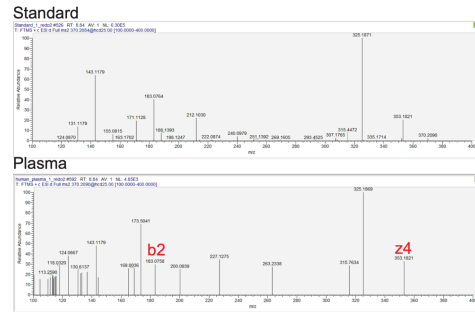
a. CAP-TAC1



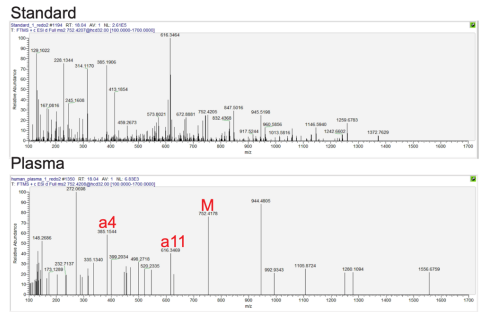
b. CAP-FGF18



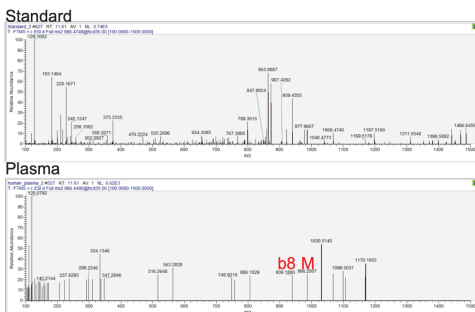
c. CAP-EMILIN1



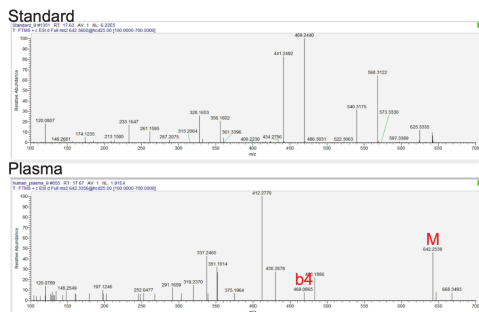
d. CAP-VIP



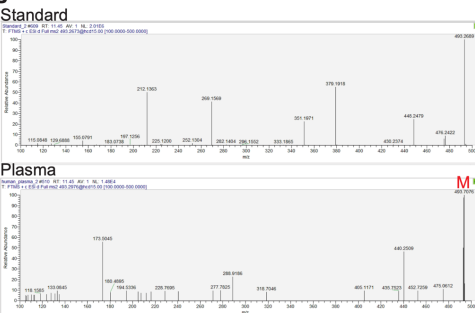
e. CAP-PMCH



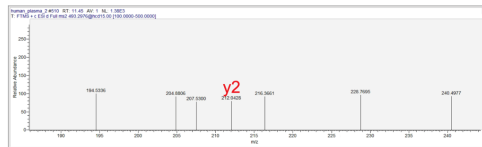
f. CAP-LGI3



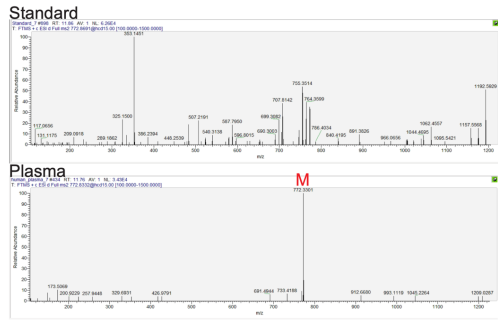
g. CAP-COL27A1



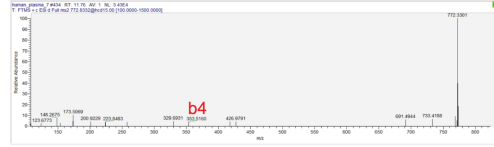
Zoomed >



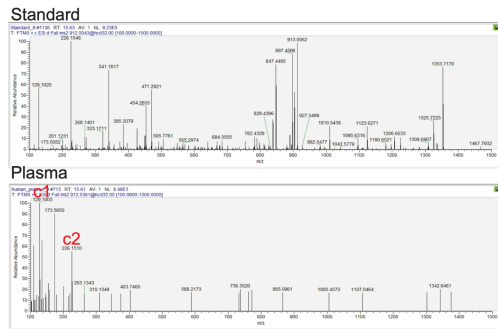
h. CAP-COL11A2



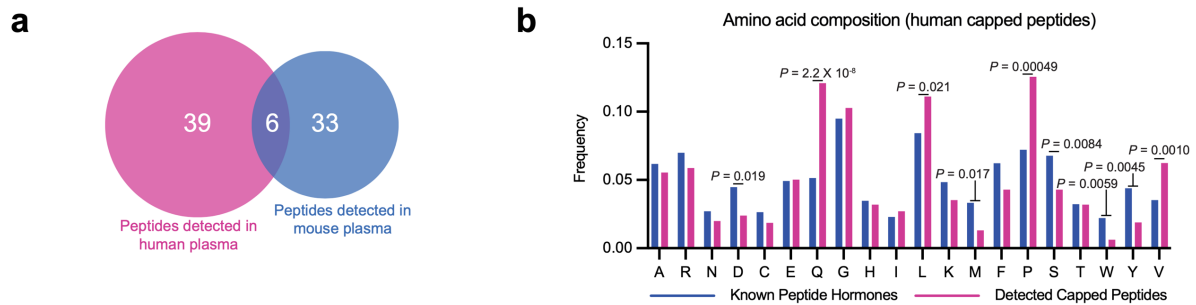
Zoomed >



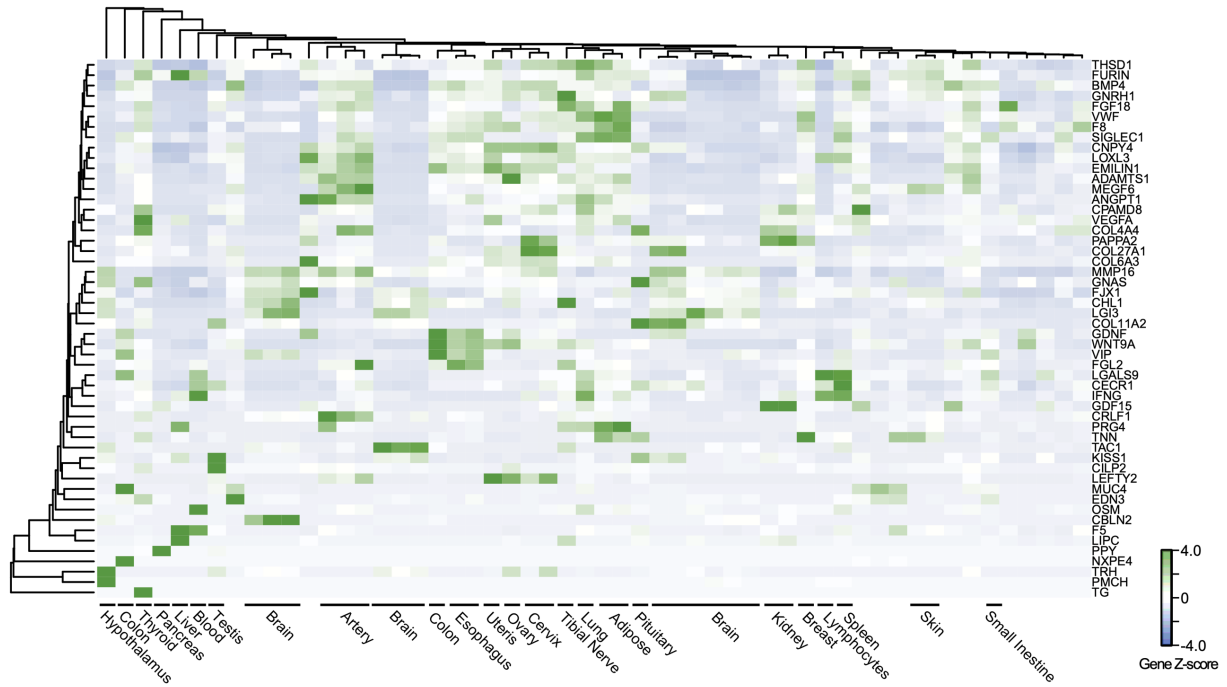
i. CAP-FURIN



Supplementary Figure 7. Additional MS2 spectra for human capped peptides. a-i MS2 spectra of 9 human capped peptides both in human plasma and of the synthetic standards.



Supplementary Figure 8. Prediction, detection, and composition analysis of human capped peptides. **a** Venn diagram showing the amount of peptides detected in human and mouse plasma and overlap between the two. **b** Comparison of frequency of each amino acid between human capped peptides and known peptide hormones. For **(b)**, data is presented as mean of frequency, and P-value versus control was calculated by two-sided Student's t-test. Source data are provided as a Source Data file.



Supplementary Figure 9. Tissue distribution of mRNAs for home genes corresponding to human capped peptide. H-clustered heat map of mRNA expression for capped peptide preprop recursor home genes across human tissues and cell types, using GTEx as the reference database. Source data are provided as a Source Data file.